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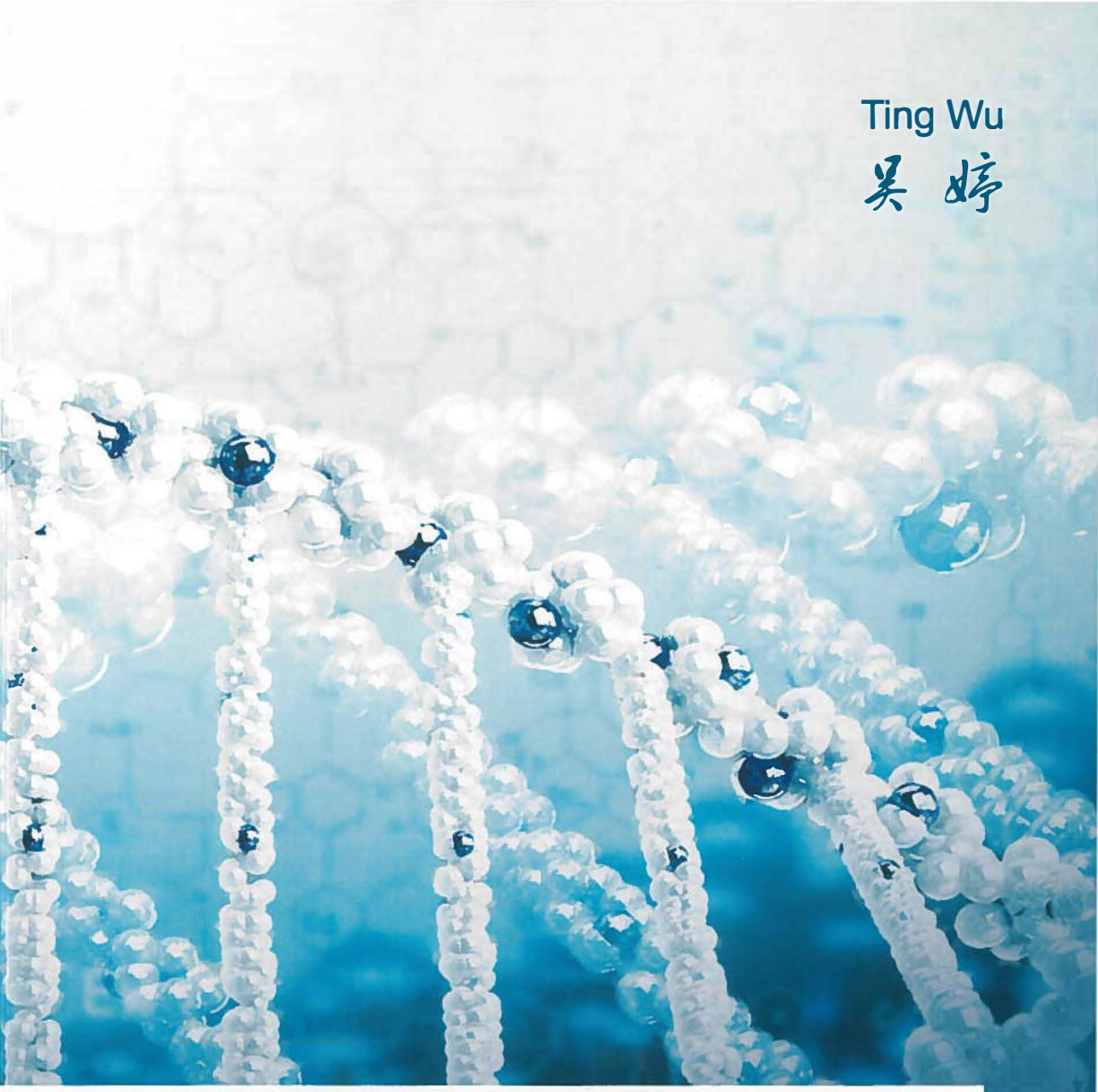

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Propositions

1. The etiology of most common diseases involves not only discrete genetic and environmental causes, but also interactions between the two. *(Hunter, Nature Reviews Genetics 2005)*
2. CYP1A1 and EPHX1 genotypes modified the association between maternal passive smoking and infant birth weight, suggesting gene-environment interaction. *(this thesis)*
3. Twin studies allow exploration of potential overall G×E effects before specific genes have been identified, which may provide clues for subsequent gene-finding studies. *(this thesis; Thomas, Nature Reviews Genetics 2010)*
4. Bivariate genetic modeling in twins showed that cardiovascular measures obtained during rest and stress show substantial heritability that is comparable between individuals of African and European descent. *(this thesis)*
5. Genetic effects explain the majority of the variation in objective intermediate asthma phenotypes. *(this thesis)*
6. Individual differences in both urinary excretion rates of norepinephrine and epinephrine and the association between them are substantially heritable without ethnic and gender effects. *(this thesis)*
7. Stay hungry, stay foolish. *(Steve Jobs)*
8. Good company on the road is the shortest cut. *(Proverbs)*
9. Don't spend time beating on a wall, hoping to transform it into a door. *(Coco Chanel)*
10. There are two sides to every question. *(Proverbs)*

Propositions belonging to the thesis "Influence of Genes, Environment and their Interaction on Risk Factors for Asthma and Cardiovascular Disease" to be defended by Ting Wu on Wednesday 6 November, 2013 in Groningen, The Netherlands.

cmb.

RIJKSUNIVERSITEIT GRONINGEN

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CONTENTS

CHAPTER 1

General introduction and aims of the thesis

Definition of gene-environment interaction

One of the longest, and at times most contentious, debates in Western intellectual history concerns the relative influence of genetic and environmental factors on human behavioral differences, the so-called nature-nurture debate However, behavioral genetic research has led many to conclude that it may now be time to retire this debate in favor of a perspective that more strongly emphasizes the joint influence of genes and the environment in shaping the outcome phenotypes (1, 2). Obtaining high-quality information on environment and lifestyle in conjunction with biological samples to assess these genetic variants will be crucial in the assessment of gene–environment interactions. Planning for these studies is needed now if reliable data on gene–environment interactions are to keep pace with rapidly emerging genetic knowledge (3).

An interaction is defined when two or more causes act in concert to produce or prevent an effect that is different from the effect of either factor considered alone. The interaction can be categorized as either synergistic (i.e., the combined effect of two or more factors is greater than the sum or product of their solitary effects) or antagonistic (i.e., the combined effect of two or more factors is smaller than the solitary effect of any one of the factors or sum of the individual factors). Analogously, the gene–environment interaction can be generally defined as the norm of reaction in the set of phenotypes that are associated with an individual's genotype or genome type when exposed to different environmental conditions (4, 5); that is, an increased (or decreased) risk for the disease or an increased (or decreased) genetic expression when carriers of a certain genotype are exposed to certain environmental factors compared to noncarriers. In particular, this interaction consists of five types described by Ottman in 1996 (6) as follows: 1) when the presence of a genotype produces or increases the effect of the environmental variable. In other words, the gene acts on the pathway to which the environmental factor belongs; 2) when a genotype magnifies the effects of an environmental variable, but in the absence of the environmental variable the genotype imposes no risk; 3) the environmental variable intensifying the effect of the genotype, but the environmental effect is minimal to none in individuals with a low-risk genotype; 4) when both the genotype and the environmental variable are required to produce the increased risk of the phenotype; and 5) when the genotype or the environmental variables individually increase the risk of the phenotype, but the risk increases even more when they occur together.

The search for genetic factors that influence common, complex traits and the characterization of the effects of those factors is both a goal and a challenge for modern geneticists (7). The first challenge is the detection of major genes that do not have estimated lifetime risks that reach 100 percent (i.e., incomplete penetrance). Incomplete penetrance may result from the role of other factors in disease etiology, such as environmental factors, that may or may not interact with the genetic factors. Second, many inconsistent associations have been found across candidate gene studies. In the last couple of years, the field for gene-finding has been revolutionized by the success of genome-wide association (GWA) studies (8-13) which have identified hundreds of genetic variants associated with complex human diseases and traits, and have provided valuable insights into their genetic architecture. However, most variants identified so far confer relatively small increments in risk, and explain only a small proportion of phenotype variance, leading many to question how the remaining, 'missing' heritability can be explained (14, 15). This unexplained genetic variation may be due to low frequency alleles or other types of variation not captured by current GWAS techniques and/or to underdeveloped data analysis methods for detecting complex interaction(14, 16). The detection of complex interaction such as gene-environment interaction is important because if a genetic factor operates primarily through a complex mechanism involving multiple other genes, and possibly environmental factors, the effect may be missed if one examines it in isolation, without allowing for its potential interactions with these other (unknown) factors (7).

The most commonly used study designs hitherto to explore gene-environment interaction can generally be divided into two categories: designs in which DNA is measured, for example, candidate gene studies, and designs in which DNA is unmeasured, for example, twin and family studies.

Twin studies: Variance Components Approaches

In family studies, the genetic relatedness is confounded with the shared environment of the family members. This includes potentially important sources of inter-individual variance like culture, socioeconomic status (SES), neighborhood, school, sports club, peers, family diet, and parental rearing style and attitudes. A unique experiment of nature has provided the solution to separating genetic and shared environmental influences: the existence of monozygotic (MZ) and dizygotic (DZ) twins (17).

Twin methodology is concerned with analysis techniques that have been developed to

estimate and quantify the relative contribution of genes and environment to the disease or trait of interest (18). The classic twin study builds on the biological fact that there are, genetically, two kinds of twins that provide contrasting degrees of genetic relationship in siblings of the same age and family circumstances. Monozygotic (MZ) twins share identical genotypes; therefore, any differences between them are due to their environments. Dizygotic (DZ) twins, in contrast, are no more alike genetically than siblings, sharing on average 50% of their segregating genes. If it is assumed that both types of twins share environmental influences to the same extent, any greater similarity in attributes among MZ twins when compared with DZ twins reflects the importance of genetic influences. The assumption of equal environmental sharing in MZ and DZ twins has been frequently criticized as a potential weakness of the twin design. However, studies specifically carried out to test it (e.g. studies conducted among twins where zygosities had been misassigned) have shown no instances where violation of this assumption leads to important bias in interpretation of the results of classic twin studies (18, 19).

Twin studies not only provide estimates of the relative contribution of genetic and environmental factors, but also allow exploration of gene-environment interactions. This interaction can be evaluated by three kinds of approaches. First, the phenomenon of 'environmental sensitivity' refers to a situation in which a gene's effect is mediated not through an influence on the level of a measured phenotype, but rather through its variability in response to the environment. This can be approached directly through variance components analyses of responses to specific environmental challenges such as allergens or stressors. In this approach, the structural equation modeling (SEM) used is based on the comparison of the variance-covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into its genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components (**Figure 1**) (20, 21). For example, Thomsen et al. (22) used a large sample of Danish adult twins to study the genetic influences on skin prick test (SPT) reactivity to different allergens and airway or bronchial hyperresponsiveness (BHR) as measured by PC_{20}, defined as the concentration of saline inhalation causing a 20% fall in Forced Expiratory Volume in one second (FEV_1). The heritability for SPT reactivity and BHR were 0.85 and 0.57, respectively. Similarly, Ferreira et al. (23) performed SEM in Australian twins and families and found that the heritability of SPT reactivity and BHR were 0.49 and 0.58, respectively. These studies suggested that the genetic factors play an important role in

some allergic phenotypes especially when environmental challenges exist. Heritabilities of such traits inherently represent gene-environment interactions. Such results may provide guidance to future gene finding studies and point to the relevance of measuring environmental factors more rigorously.

Second, with bivariate path models, making use of the 'Cholesky decomposition', we can simultaneously model the rest level and the response to a specific environmental challenge (e.g., a mental stress task) (17, 24). The variance in the observed traits (e.g., SBP at rest and SBP during stress) is decomposed into latent additive genetic, shared environmental and unique environmental components. The relative contribution of genetic variance to the total variance in the SBP at rest, also known as its heritability, is the effect of the genetic factor A, and obtains as the ratio of $a_{11}^2/(a_{11}^2 + c_{11}^2 + e_{11}^2)$. The heritability of SBP during stress is the summed effect of the genetic factors A and A*s*, and obtains as the ratio of genetic to total variance, or $(a_{21}^2 + a_{22}^2)/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. When going from rest to stress, the effects of the genetic differences between subjects may be amplified ($a_{21} > a_{11}$) or dampened ($a_{21} < a_{11}$) by the stressors. In addition, entirely new genetic variation between subjects may emerge only during stress, depicted by factor A*s*. In this case, the path-coefficient a_{22} will differ significantly from zero ($a_{22} > 0$). This part of the total heritability of the stress level represents the influence of novel genetic effects only expressed during stress and is equal to $a_{22}^2/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. Both amplification/dampening and emergence effectively constitute forms of gene-by-stress interaction (**Figure 2**) (25, 26).

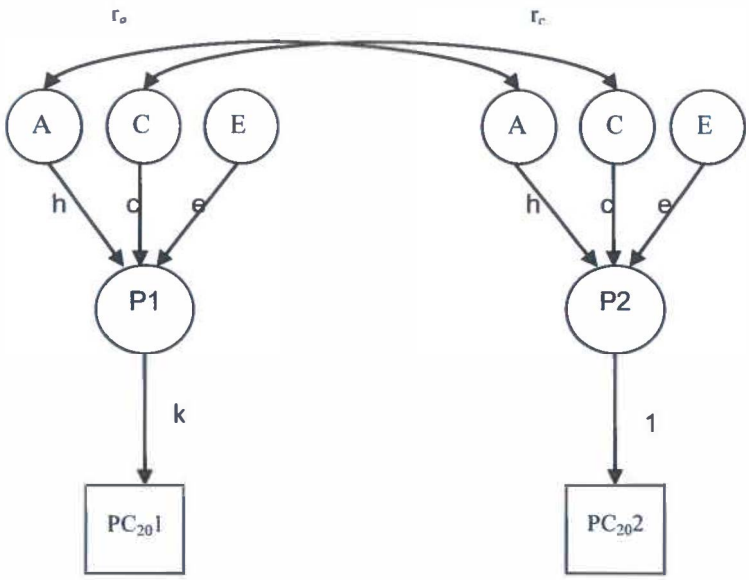

Figure 1. Path diagram for a univariate model. An opposite-sex twin pair is shown, twin 1 being male and twin 2 female. Observed phenotypes (P) for twin 1 and twin 2 are shown in squares (here we used PC_{20} as an example), latent (i.e. unmeasured) factors are shown in circles. r_g is the correlation between additive genetic factors, which is 1 in MZ twins, 0.5 in DZ same sex twins, while r_c is the correlation between common environmental factors which is 1 in MZ twins and DZ same sex twins. r_g and r_c can be estimated for dizygotic twin pairs of opposite sex. Regression coefficients of observed variables on the different latent factors are shown in lower case: h = additive genetic effect, c = common environmental effect, e = unique environmental effect, k = scalar factor. D = dominance genetic influence, was also tested but is omitted to simplify the diagram.

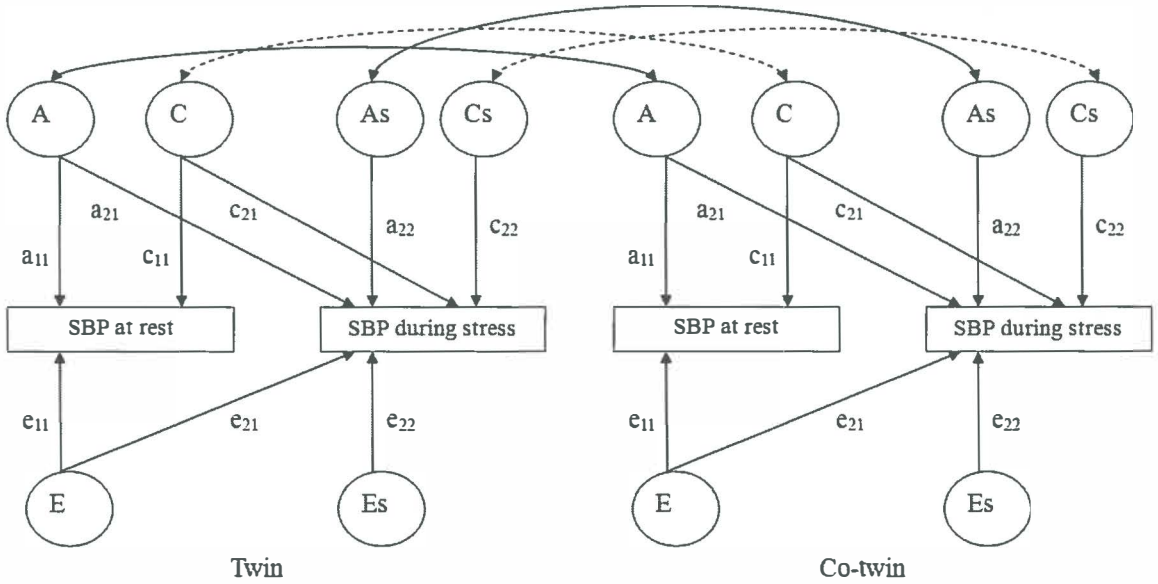

Figure 2. Bivariate twin model for genetic and environmental influences on systolic blood pressure (SBP). Biometrical genetic theory specifies that the additive genetic factors (denoted by A and A*s*) of (monozygotic) MZ twins are perfectly correlated (1.0), whereas those of dizygotic (DZ) twins are correlated 0.5. Common environmental factors shared by twins from the same family (denoted by C and C*s*) are correlated unity for both types of twins, whereas the unique environmental influences (E and E*s*) are always uncorrelated. Path coefficient a*11* quantifies the effect of genetic influence A on SBP at rest, a*21* quantifies the effect of A on SBP during stress, and a*22* quantifies the effect of emergent genes in A*s* on SBP during stress. In a similar way, path coefficients e*11*, c*11*, e*21*, and c*21* quantify the effects of common and unique environmental influences E and C on SBP at rest and during stress. e*22* and c*22* quantify the effect of emergent environmental influences in E*s* and C*s* on SBP during stress

Third, gene-environment interaction SEM as introduced by Purcell (27) directly incorporates the environmental factor as a continuous moderator into the model and allows to estimate whether and to what extent the A (or D), C and E components on a trait of interest are modified by the specific environmental factor. In this gene-environment interaction model, the phenotypic variance of the outcome variables is portioned into A, C, and E components with the path coefficients associated with each variable expressed as linear functions of the moderator (e.g., A+T×M1, C+U×M1, E+V×M1) where M1 represents the value of the moderator (e.g., a measured environmental factor) and B represents linear effects on the outcome (**Figure 3**). McCaffery et al. (28) performed twin SEM to investigate the potential for gene-environment interaction in hypertension by examining the extent to which educational attainment modifies the heritability of hypertension in a large sample of male Vietnam-era twins. Moderation of additive genetic effects on hypertension by education level was found and the results illustrated greater heritability of self-reported hypertension at higher levels of educational attainment, or gene x educational attainment interaction, showing that a 4-year difference in educational attainment was associated with an 8-point increase in heritability from 0.53 to 0.61. The results indicated that the expression of genetic vulnerability to hypertension can vary as a function of environmental factors and that nongenetic pathways may differentially contribute to risk among those with fewer years of education.

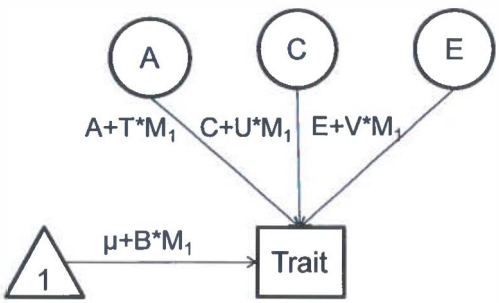

Figure 3. Partial path diagram for the basic gene-environment interaction model. A=additive genetic effects; C=common environmental effects; E=unique environmental effects; M=moderator (e.g., educational attainment); T=moderated component of A; U=moderated component of C; V=moderated component of E; B=linear effects of moderator on mean (forced entry)

Candidate gene studies

Any of the standard epidemiological designs for studying the main effects of genes or environmental factors - cohort designs, case-control designs or hybrid designs, such as nested case-control designs or case-cohort designs- can be applied to the study of G×E interactions. In addition, family-based association tests and some novel designs like two-phase case-control and counter-matching designs can improve the power for detecting either main effects or interaction(15). The multifactor dimensionality reduction (MDR) design and gene-set-enrichment analysis allow a set of candidate genes to be studied together to learn about the overall effect of the postulated pathway(s)(7, 15).

The case-control study design using unrelated controls is the most commonly used design in studies of gene-environment interaction (29). Using unexposed subjects with no susceptibility genotype as the reference group, odds ratios for all other groups can be estimated. Adjustment for potential confounding variables may be done by using stratification or multivariate approaches (30). The main advantage of this design is that the main effects of the environmental exposure and genetic susceptibility, as well as their interactive effect, may be estimated. The main disadvantage is that this retrospective design may not be appropriate for the study of gene-environment interaction involving rare genes or uncommon environmental exposures (assuming moderate values of the interaction effect) as environmental exposures are inaccurately measured (31).

Another commonly used design for candidate gene studies is the cohort study. Cohort studies are follow-up studies of disease incidence in which a group of individuals are classified according to their exposure status for an environmental factor (e.g., exposed versus unexposed). In cohort studies one is interested to learn whether exposure to a 'risk factor' is associated with developing a disease. Since, individuals in a cohort are not selected based on exposure or disease status; such a study can be used to estimate exposure prevalence, disease prevalence, and the association between them. This design has important strengths in characterizing exposures and risk factors before disease onset, which reduces selection and recall biases that are common in case–control studies. Here, DNA samples and exposure information are obtained from participants in a longitudinal cohort who are followed up, usually for years or decades. If follow-up rates are high, then a virtually complete set of cases can be assembled and compared with a sample of individuals who did not develop the disease (3).

Furthermore, the prospective cohort design is preferable for studies of common diseases that seem to be genetically complex, that is, due to many genes of small effect rather than a single major gene. This is because the breadth and reliability of the environmental exposure data that can be obtained prospectively allows the examination of key gene–environment interactions and, consequently, greater validity in estimates of genetic effects. This and other strengths of prospective cohort studies make them invaluable for understanding gene–environment interactions in complex human disease (31). Whatever study design is used, the major challenges to the success of a G×E study — in addition to the usual challenges for genetic association studies that have been thoroughly discussed elsewhere — are exposure assessment, sample size and heterogeneity(15).

Scope and content of the thesis

The present thesis aims to apply different gene-environment interaction methods as discussed above to a wide range of human traits and diseases. It starts with a prospective candidate gene study, which aims to give more insight in interaction between maternal passive smoking and maternal metabolic genes on infant birth weight. The greater part of this thesis, however, consists of several twin studies appearing in subsequent chapters. These have the purpose to improve and extend the application of twin modeling to explore gene-environment interactions, Univariate SEM was performed in chapter 3 to examine heritabilities of responses to environmental challenges, such as BHR and SPT. Similarly, in chapter 4, heritabilities of overnight urinary excretion rates of norepinephrine ($U_{NE}V$) and epinephrine (U_EV), which reflect basal sympathetic activity levels as a measure of chronic exposure to stress, were explored by the same twin modeling. A Meta-analysis that pooled the results of all published twin studies on heritablities of heart rate (HR) or BP reactivity to the cold pressor test or various mental stress tasks was presented in chapter 5. In chapter 6, we used bivariate modeling to estimate the contribution of genes and environment to the individual differences in levels of BP and underlying hemodynamic characteristics at rest and during stress. Finally, in chapter 7, Purcell's GxE model was applied to examine the extent to which BMI as a measure of adiposity and treated as an environment factor in this model, may modify the genetic influence on BP.

In **chapter 2,** we aimed to determine whether polymorphisms in two maternal metabolic genes, cytochrome P-450 1A1 (CYP1A1) MspI and epoxide hydrolase 1 (EPHX1) Tyr113His, affect the association of maternal passive smoking with infant birth

weight in a cohort of 1388 (680 nonpassive smokers and 708 passive smokers) newly married mothers in China. This analysis was part of a prospective reproductive health study carried out from 1996 to 2000 among female textile workers. The Chinese marriage registration system was used to identify newly wed couples and those planning a first pregnancy. Information on passive smoking during the index pregnancy was based on women's self-reporting and was obtained for three time periods: the first, second, and third trimesters. Each woman recorded the mean number of cigarettes smoked per day at home by regular household members during the three time periods.

In **chapter 3**, we examined heritabilities of objective asthma-related traits, such as baseline lung function (FEV_1, Forced Vital Capacity (FVC) and FEV_1/FVC), BHR, number of positive SPTs to eleven common allergens and number of positive specific Immunoglobulin E (IgE) tests to four allergens, and estimated their environmental and genetic overlap. The participants consisted of 206 twins from 46 monozygotic and 57 dizygotic pairs (mean age: 22.5 years, range: 17.0 - 27.0). Univariate and bivariate genetic analyses were performed after adjustment for significant covariates. The study population consisted of twins who participated in the Netherlands Twin Register (NTR) study of health related behavior. The NTR represents a random sample of Dutch families with twins, recruited from city council registrations. A total of 102 families participated in the current study contributing 103 twin pairs.

In **chapter 4**, genetic and environmental contributions to overnight urinary excretion rates of norepinephrine ($U_{NE}V$) and epinephrine (U_EV) as measures of basal sympathetic activity, and their association with BP were investigated in 91 African American (AA) and 101 European American (EA). NE and E mediate the stress response via the sympathetic nervous system. This study comprised 101 EA and 91 AA twins from the Georgia Cardiovascular Twin Study. Overnight urine volume data was used to estimate $U_{NE}V$ and U_EV.

In **chapter 5**, we performed a meta-analysis on all published twin studies that assessed HR or BP reactivity to the cold pressor test or various mental stress tasks. Moreover, we also briefly listed the heritability estimates of a number of other cardiovascular measures for which sufficient numbers are not yet available to do a meta-analysis. We further reviewed the first attempts to find genetic associations with reactivity measures in molecular genetic studies. This study, therefore, consisted of a comprehensive summary of gene-environment interactions with respect to the heritability of cardiovascular reactivity to specific stressful environmental challenges. However, a

limitation of most twin studies performed so far, and hence of the meta-analysis based on these studies, is that they analyzed reactivity as a change score, which can not test newly emerging genetic or environmental influences during stress and amplification or dampening of genetic or environmental influences already present at rest. Thus in **chapter 6**, we further performed bivariate modeling of resting and aggregated stress levels to explicitly test for emergence and amplification.

In **chapter 6**, we estimated the contribution of genes and environment to the individual differences in levels of BP and underlying hemodynamic characteristics at rest and during stress using a bivariate approach in a large twin cohort of youth. We further examined ethnic differences as very few previous studies included both EA and AA twins. This study comprised 308 EA and 226 AA twin pairs from the southeastern United States and part of the Georgia Cardiovascular Twin Study, which was established in 1996.

In **chapter 7**, a twin study was performed to examine to what extent the relation between BP and body mass index (BMI) can be explained by genetic or environmental factors and the extent to which BMI may modify the genetic influence on BP. This study included 1243 monozygotic and 833 dizygotic Han Chinese twins recruited from Qingdao and Lishui located in the north and south of China, respectively, from the Chinese National Twin Registry (CNTR) established in 2001.In **chapter 8**, the general discussion, the studies in this thesis were put into a broader perspective. In this chapter we discussed the research findings and brought up methodological considerations. The implications and perspectives for future research were also explored. Finally, the thesis was concluded with a summary and some final remarks.

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CHAPTER 2

Passive smoking, metabolic gene polymorphisms, and infant birth weight in a prospective cohort study of Chinese women

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Original Contribution

Passive Smoking, Metabolic Gene Polymorphisms, and Infant Birth Weight in a Prospective Cohort Study of Chinese Women

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The authors investigated whether polymorphisms in two maternal metabolic genes, cytochrome P-450 1A1 (*CYP1A1*) *MspI* and epoxide hydrolase 1 (*EPHX1*) *Tyr113His*, affect the association of maternal passive smoking with infant birth weight. The study was conducted in a cohort of 1,388 newly married mothers of liveborn singletons who worked in textile mills in Anqing, China, from 1996 to 2000. Multiple linear regression models were used to estimate the associations of passive smoking and genetic susceptibility with birth weight, with adjustment for important potential confounders. In the passive smoking group, there was a remarkable decrease in birth weight with the *C/C6235* genotype (156.3 g, 95% confidence interval (CI): −283.6, −29.0) for *CYP1A1 MspI* and with *Tyr/His113* (93.8 g, 95% CI: −188.6, −1.1) as compared with *His/His113* (244.6 g, 95% CI: −491.0, −1.9) for *EPHX1*. When results were stratified by maternal genotype, passive smoking conferred a significantly negative effect in the *EPHX1 Tyr/His113* group (103.5 g, 95% CI: −205.8, −9.2) and in the *His/His113* group (687.3 g, 95% CI: −748.3, −178.3). The data further showed that there was a significant interaction between maternal passive smoking and maternal *EPHX1* genotype for birth weight. The authors conclude that the *CYP1A1 MspI* and *EPHX1* genotypes modified the association between maternal passive smoking and infant birth weight in this study, which is suggestive of possible gene-environment interaction.

birth weight; cytochrome P-450 CYP1A1; epoxide hydrolases; infant; polymorphism, genetic; tobacco smoke pollution

Abbreviations: CI, confidence interval; CYP1A1, cytochrome P-450 1A1; EPHX1, epoxide hydrolase 1; PCR, polymerase chain reaction.

Low birth weight (<2,500 g) is a powerful predictor of infant survival and childhood morbidity, as well as adulthood health conditions (1, 2). The etiology of low birth weight remains unclear, but both environmental and genetic factors may play important roles (3). These factors may include cigarette smoking (4, 5), caffeine consumption (6), exposure to pesticides, organic solvents, and related compounds (7, 8), infant sex, prenatal maternal mood (9), and strong familial aggregation (3). Exposure to cigarette smoke during pregnancy, via active or passive routes, is known to be a strong risk factor for preterm birth and low birth weight (10–15). However, not all women who have been passively exposed to cigarettes during pregnancy have infants with reduced birth weight. This variability may be related to genetic susceptibility.

Cigarette smoke constituents, including mutagenic, neurotoxic, and fetotoxic agents, can pass through the placenta even in the early stages of pregnancy and are detected in the

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urine of newborns (4, 5). A person's ability to convert toxic metabolites of cigarette smoke to less harmful moieties is critical for minimizing their adverse health effects. Aryl hydrocarbon hydroxylase, encoded by the cytochrome P-450 1A1 (*CYP1A1*) gene, is a well-studied phase I enzyme and is particularly relevant to the metabolism of chemicals in cigarette smoke. Detoxification of these epoxides may occur through conjugation with certain endogenous functional groups such as glutathione, catalyzed by glutathione *S*-transferases (16), or by hydration, catalyzed by epoxide hydrolases in phase II. The end product becomes a stable hydrophilic compound that can easily be excreted (17). The microsomal form of epoxide hydrolase, epoxide hydrolase 1 (EPHX1), which is encoded by the *EPHX1* gene, catalyzes the conversion of a broad spectrum of highly reactive aliphatic epoxides to less toxic *trans*-dihydrodiols (18). Both the *CYP1A1* gene and the *EPHX1* gene are highly polymorphic in the population (19, 20), and their polymorphisms have been associated with their encoded enzyme activities. *CYP1A1 Msp*I variance may increase aryl hydrocarbon hydroxylase activity, and *EPHX1 Tyr113His* variance may decrease EPHX 1 activity (21, 22). Interindividual differences in susceptibility to the adverse health effects of cigarette smoke are partly attributable to different maternal genotypes associated with these enzymes.

Previous studies have shown that maternal genotype can modify the association between maternal cigarette smoking and infant birth weight (23, 24), and 70 percent of nonsmoking Chinese women between the ages of 20 and 50 years have reported passive exposure to cigarette smoke (25). It is important to elucidate the effects of passive smoking on pregnancy outcomes; even if the magnitudes of effect are modest, the adverse impacts on the public's health will be widespread with so prevalent an exposure. In this study, we used *CYP1A1 Msp*I and *EPHX1 Tyr113His* gene polymorphisms to characterize genetic susceptibility to low birth weight and to assess the interaction between metabolic genes and passive cigarette smoking.

MATERIALS AND METHODS

Study site and population

This analysis was part of a prospective reproductive health study carried out from 1996 to 2000 among female textile workers in Anqing, China, an urban area approximately 200 km west of Shanghai. All employees of the textile mills received health care, including prenatal care, delivery, and postnatal care, at Anqing Hospital. To be eligible for inclusion during field enrollment, an employee had to be 1) working full-time, 2) newly married, and 3) aged 20–34 years and 4) had to have obtained permission to have a child. All of the women were nulliparous. Women were excluded if 1) they were already pregnant before enrollment; 2) they had tried unsuccessfully to get pregnant for at least 1 year at any time in the past; or 3) they were planning to quit, change jobs, or move out of the city over the 1-year course of follow up. The study protocols were approved by the Human Subject Committee of Beijing Medical University. We obtained written informed consent from each woman.

Data collection procedures

We used the Chinese marriage registration system to identify newlywed couples and those planning a first pregnancy. Upon enrollment, a physical examination was performed, and height and weight were measured according to a standard protocol. At enrollment, a structured baseline questionnaire was administered by a trained interviewer to the women and their husbands in order to collect information on occupational exposures, personal habits such as cigarette smoking and alcohol consumption, living environment, passive exposure to cigarette smoke, dietary intake, menstrual and reproductive history, and contraceptive use. When a woman decided to stop contraception in order to become pregnant, she began keeping a daily diary on menstrual bleeding and associated symptoms and exposure to tobacco smoke and other occupational exposures. If a woman reported a missed or late menstrual period or had early signs or symptoms of pregnancy, she was instructed to go to Anqing Hospital for a check-up and to give a urine sample for pregnancy testing. Once a woman was confirmed to be pregnant, collection of the daily diaries was terminated and the woman received regular prenatal care and delivery services at the designated hospital according to standard clinical guidelines. The woman was followed up with regard to pregnancy outcomes, including infant birth weight, gestational age, and infant sex, by the research staff. In this study, infant birth weight was measured in the delivery room by a trained nurse and was accurate to 1 g. Blood samples were obtained from women via venipuncture by a skilled phlebotomist. The genomic DNA was extracted according to a standard protocol (26).

Assessment of environmental tobacco smoke

There is evidence showing that cotinine level is quite positively related to self-reported passive smoke exposure (27, 28). Therefore, in this study, information on passive smoking during the index pregnancy was based on women's self-reporting and was obtained for three time periods: the first, second, and third trimesters. Each woman recorded the mean number of cigarettes smoked per day at home by regular household members during the three time periods. In our study, the specific question on the questionnaire was, "On average, what is the number of cigarettes someone smoked indoors at home per day while you were exposed in the last 3 months?" Exposure to tobacco smoke at the workplace was not considered, because none of the employees at the textile mills were allowed to smoke at work. In the subsequent analysis, maternal passive smoking at home was considered as both a binary variable and a continuous variable. We defined "non-passive smoking" as no smoking by regular household members at home during any of the three time periods of pregnancy and "passive smoking" as any smoking by regular household members at home during any of the three time periods. We calculated the average number

of cigarettes smoked per day at home during the three time periods.

Genotyping methods

Detection of the CYP1A1 MspI polymorphism. For detection of the *CYP1A1 Msp*I polymorphism, the primers used in the polymerase chain reaction (PCR) were bases 42–65 (5′-TCACTCGTCTAAATACTCACCCTG-3′) and the segment from base pair 435 to base pair 455 (5′-TAGGAGTCTTGTCTCATGCCT-3′). A 20-ng DNA sample was used in a 10-μl PCR reaction containing 50 mM potassium chloride, 1.5 mM magnesium chloride, 10 mM Tris-hydrochloric acid, 0.1 percent Triton X-100, 200 μM deoxyribonucleoside triphosphates, 200 nM primers, and 0.25 U Taq polymerase. The PCR amplification was carried out under the following conditions: 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 7 minutes. The reaction products were subjected to digestion by *Msp*I for 15 hours at 37°C. This process results in 295-, 160-, and 135-base-pair products and is able to detect all three possible genotypes for the polymorphism: *T/T6235* (homozygous wild type), *T/C6235* (heterozygous variant type), and *C/C6235* (homozygous variant type).

Detection of the EPHX1 Tyr113His polymorphism. We examined the *EPHX1 Tyr113His* polymorphism at position 113 in exon 3 of the *EPHX1* gene. We used the primers 5′-GGCTTCAACTCCAACTACCTG-3′ and 5′-CAATCTTAGTCTTGAAGTGACGGT-3′ in the PCR. A 20-ng DNA sample was used in a 10-μl PCR reaction containing 50 mM potassium chloride, 1.5 mM magnesium chloride, 10 mM Tris-hydrochloric acid, 0.1 percent Triton X-100, 200 μM deoxyribonucleoside triphosphates, 200 nM primers, and 0.25 U Taq polymerase. The PCR amplification was carried out at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 7 minutes. The products of 112 base pairs were digested with *Tth111*I for 15 hours at 37°C. Persons with the homozygous wild genotype show 30- and 90-base-pair fragments, while heterozygous persons show three bands at 30, 90, and 120 base pairs, respectively. Persons with the homozygous variant genotype show only a 120-base-pair band.

Previously sequenced genomic DNA samples were used as positive controls for the homozygous wild, heterozygous, and homozygous mutant genotypes with every PCR analysis to verify the reproducibility of the restriction fragment length polymorphism PCR and to confirm the accuracy of genotype classification. Approximately 10 percent of the samples were randomly selected for reanalysis for verification of the results of the genotyping assays.

Statistical methods

Considering the low number of low birth weight infants (6.5 percent for the nonexposed group and 9.6 percent for the exposed group) and to preserve statistical power, we analyzed our data using birth weight as a continuous variable. We first examined the association between maternal genotype and infant birth weight without consideration of passive cigarette smoking, using multiple linear regression modeling with adjustment for major covariates. We then investigated whether the association between passive smoking and reduced birth weight was modified by maternal genotype, by estimating the association between passive smoking and birth weight in total samples as well as in subgroups stratified by the specific maternal genotypes. To further assess gene-environment interaction, we examined the combined association of passive smoking and maternal genotype with birth weight in six subgroups defined by passive smoking status (no, yes) and maternal genotype for *CYP1A1 Msp*I (*T/T6235*, *T/C6235*, and *C/C6235*) and *EPHX1* (*Tyr/Tyr113*, *Tyr/His113*, and *His/His113*). Finally, we tested for gene-environment interaction by adding a product term in the regression model. In all analyses, results were adjusted for the following important potential confounders: maternal age (<25, 25–27, and ≥28 years), education (elementary school, middle school, and high school or above), shift work (no, yes), exposure to noise at work (no, yes), exposure to vibration at work (no, yes), whether the job was perceived to be stressful (no, yes), prepregnancy height and weight, average number of cooking days per week (days on which the woman cooked for her family; <1, 1–2, 3–5, and 6–7 days/week), history of pregnancy (prior miscarriage or abortion; no, yes), and infant sex. All p values were two-sided, and statistical significance was defined as $p = 0.05$. Selection of the covariates was based on the current literature on passive smoking and the standard statistical procedures for variable selection (23, 24). We use SAS software (SAS Institute Inc., Cary, North Carolina) for all analyses. The frequencies of the T alleles of *CYP1A1 Msp*I and the C alleles of *EPHX1 Tyr113His* in these populations conformed to Hardy-Weinberg equilibrium.

RESULTS

A total of 1,540 mothers were invited to participate in this study. Of those, 152 mothers were excluded because of failure in extracting DNA, failure of genotyping, or missing data. The final analysis included 1,388 mothers (680 nonpassive smokers and 708 passive smokers) who gave birth to live singletons at the Anqing Hospital. As table 1 shows, the nonexposed and exposed groups were similar in terms of age distribution, maternal prepregnancy weight, shift work, exposure to noise and toxins, perceived stress, cooking, and infant sex, whereas the two groups differed with regard to maternal prepregnancy height, education, exposure to vibration in the current job, and history of pregnancy. The mean birth weight was 30.5 g lower ($p = 0.2317$) for the exposed group than for the nonexposed group, but there was no significant difference. The mean gestational age was 39.5 weeks for both the exposed group and the nonexposed group.

Table 2 presents mean values and standard deviations for the total sample and for subgroups defined by maternal

TABLE 1. Characteristics of female textile mill workers and their newborn infants according to passive exposure to cigarette smoke at home during pregnancy, Anqing, China, 1996–2000

Variable	Nonexposed (n = 680)		Exposed (n = 708)		p value
	No. or mean	%	No. or mean	%	
Maternal characteristics					
Age (years)					0.085
<25	420	61.8	424	59.9	
25–27	244	35.9	252	35.6	
≥28	16	2.4	32	4.5	
Mean prepregnancy height (cm)	157.9 (4.8)*		157.2 (5.1)		0.0139
Mean prepregnancy weight (kg)	49.6 (5.5)		49.3 (5.7)		0.2713
Education					0.003
Elementary school	12	1.8	8	1.1	
Middle school	472	68.4	548	77.4	
High school or above	196	28.8	152	21.5	
Exposure to vibration in the current job					0
No	536	78.8	492	69.5	
Yes	144	21.2	216	30.5	
Shift work					0.504
No	36	5.3	32	4.5	
Yes	644	94.7	676	95.5	
Exposure to noise in the current job					0.058
No	228	33.5	204	28.8	
Yes	452	66.5	504	71.2	
Current job perceived as stressful					0.087
No	392	57.6	440	62.1	
Yes	288	42.4	268	37.9	
Cooking for the family (no. of days/week)					0.4393
<1	288	42.4	292	41.2	
1–2	140	20.6	144	20.3	
3–5	172	25.3	168	23.7	
6–7	80	11.8	104	14.7	
History of pregnancy (miscarriage or abortion)					0.033
No	596	87.6	592	83.6	
Yes	84	12.4	116	16.4	
Mean amount of passive smoking (no. of cigarettes/day)	0.0 (0.0)		9.4 (9.7)		0
Infant characteristics					
Mean birth weight (g)	3,123.2 (442.8)		3,092.7 (506.5)		0.2317
Low birth weight (<2,500 g)					0.0322
No	636	93.5	640	90.4	
Yes	44	6.5	68	9.6	
Mean gestational age (weeks)	39.5 (1.6)		39.5 (1.7)		0.8302
Preterm birth (<37 weeks)					0.4621
No	644	94.7	664	93.8	
Yes	36	5.3	44	6.2	
Sex					0.759
Male	344	50.6	364	51.4	
Female	336	49.4	344	48.6	

* Numbers in parentheses, standard deviation.

TABLE 2. Effect of maternal cytochrome P-450 (*CYP1A1*) *MspI* and epoxide hydrolase 1 (*EPHX1*) *Tyr113His* genotypes* on infant birth weight, in total and by passive exposure to cigarette smoke at home during pregnancy, Anqing, China, 1996–2000

Genotype	No. of participants	Mean infant birth weight (g)	Adjusted† β‡	95% confidence interval	*p* value
Total sample					
CYP1A1 MspI					
T/T6235	492	3,123.1 (495.9)§	0.0		
T/C6235	768	3,099.8 (460.3)	−8.0	−59.6, 43.6	0.762
C/C6235	128	3,095.3 (496.9)	−63.8	−153.7, 26.0	0.164
EPHX1					
Tyr/Tyr113	1,080	3,119.2 (488.0)	0.0		
Tyr/His113	276	3,070.3 (435.5)	−60.0	−119.7, −0.3	0.042
His/His113	32	3,040.0 (402.8)	−167.9	−329.6, −6.1	0.003
Passive smoking at home					
No					
CYP1A1 MspI					
T/T6235	224	3,120.7 (452.3)	0.0		
T/C6235	404	3,123.1 (438.4)	12.6	−55.0, 80.2	0.715
C/C6235	52	3,134.6 (443.0)	1.1	−125.9, 128.1	0.986
EPHX1					
Tyr/Tyr113	512	3,119.0 (455.7)	0.0		
Tyr/His113	152	3,129.2 (406.1)	−52.3	−158.7, −17.6	0.171
His/His113	16	3,200.0 (363.3)	54.4	−161.0, 269.9	0.620
Yes					
CYP1A1 MspI					
T/T6235	268	3,125.1 (530.5)	0.0		
T/C6235	364	3,073.8 (482.7)	−56.8	−134.5, 20.9	0.152
C/C6235	76	3,068.4 (531.9)	−156.3	−283.6, −29.0	0.016
EPHX1					
Tyr/Tyr113	568	3,119.3 (515.7)	0.0		
Tyr/His113	124	2,998.1 (460.6)	−93.8	−188.6, −1.1	0.006
His/His113	16	2,880.0 (385.6)	−244.6	−491.0, −1.9	0.005

* *Tyr/Tyr113* indicates the homozygous wild type; *Tyr/His113* indicates the heterozygous variant type; and *His/His113* indicates the homozygous variant type. *T/T6235* indicates the homozygous wild type; *T/C6235* indicates the heterozygous variant type; and *C/C6235* indicates the homozygous variant type.

† Results from a multiple linear regression model with adjustment for age (<25, 25–27, and ≥28 years), education (elementary school, middle school, and high school or above), shift work (no, yes), exposure to noise in the current job (no, yes), exposure to vibration in the current job (no, yes), perceived stress of the current job (no, yes), prepregnancy weight and height (linear and quadratic terms), cooking days, history of pregnancy (no, yes), and infant sex.

‡ β represents the difference in mean birth weight between the variant genotype and the homozygous wild type after adjustment for the covariates listed above.

§ Numbers in parentheses, standard deviation.

passive smoking status. In addition, table 2 shows the adjusted association between maternal genotype and infant birth weight, where β represents the difference in mean birth weight between the variant-type group and the homozygous wild-type group after adjustment for the covariates listed. For *CYP1A1 MspI* genotypes, without consideration of passive smoking status, there was no significant effect of the *T/C6235* or *C/C6235* genotype on infant birth weight in comparison with the *T/T6235* genotype (table 2). When maternal passive smoking was considered, the association between genotype and birth weight varied remarkably by passive smoking status. In the passive smoking group, a significant decrease in mean birth weight (156.3 g, 95 percent confidence interval (CI): −283.6, −29.0) was observed among

TABLE 3. Mean infant birth weight according to maternal passive smoking at home during pregnancy and adjusted association between passive smoking and infant birth weight, in total and by maternal cytochrome P-450 (*CYP1A1*) *Msp*I and epoxide hydrolase 1 (*EPHX1*) genotype,* Anqing, China, 1996–2000

Genotype	Passive smoke exposure at home during pregnancy				Adjusted† β‡	95% confidence interval	*p* value
	No passive smoking		Passive smoking				
	No. of participants	Mean birth weight (g)	No. of participants	Mean birth weight (g)			
Total sample	680	3,123.2 (442.8)§	708	3,092.7 (506.5)	−17.2	−65.0, 30.6	0.480
*CYP1A1 Msp*I							
T/T6235	224	3,120.71 (452.33)	268	3,125.15 (530.47)	15.0	−71.2, 101.2	0.732
T/C6235	404	3,123.12 (438.44)	364	3,073.85 (482.66)	−10.5	−71.2, 50.3	0.735
C/C6235	52	3,134.62 (442.98)	76	3,068.42 (531.91)	13.6	−182.8, 209.9	0.892
EPHX1							
Tyr/Tyr113	512	3,119.02 (455.71)	568	3,119.33 (515.75)	1.6	−53.6, 56.8	0.954
Tyr/His113	152	3,129.21 (406.1)	124	2,998.06 (460.56)	−103.5	−205.8, −9.2	0.047
His/His113	16	3,200.00 (363.32)	16	2,880.00 (385.64)	−687.3	−748.3, −178.3	<0.001

* *Tyr/Tyr113* indicates the homozygous wild type; *Tyr/His113* indicates the heterozygous variant type; and *His/His113* indicates the homozygous variant type. *T/T6235* indicates the homozygous wild type; *T/C6235* indicates the heterozygous variant type; and *C/C6235* indicates the homozygous variant type.

† Results from a multiple linear regression model with adjustment for age (<25, 25–27, and ≥28 years), education (elementary school, middle school, and high school or above), shift work (no, yes), exposure to noise in the current job (no, yes), exposure to vibration in the current job (no, yes), perceived stress of the current job (no, yes), prepregnancy weight and height (linear and quadratic terms), cooking days, history of pregnancy (no, yes), and infant sex.

‡ β represents the difference in mean birth weight between passive smoking and no passive smoking in each row after adjustment for the covariates listed above.

§ Numbers in parentheses, standard deviation.

mothers with the *C/C6235* genotype as compared with those with the *T/T6235* genotype. However, a different pattern emerged for *EPHX1*. Without consideration of passive smoking status, the mean birth weight was associated with genotype; that is, compared with *Tyr/Tyr113* mothers, infant birth weight was reduced by 60.0 g (95 percent CI: −119.7, −0.3) and 167.9 g (95 percent CI: −329.6, −6.1) among *Tyr/His113* and *His/His113* mothers, respectively; in the presence of passive smoking, infant birth weight was reduced by 93.8 g (95 percent CI: −188.6, −1.1) in *Tyr/His113* mothers and 244.6 g (95 percent CI: −491.0, −1.9) in *His/His113* mothers.

Table 3 presents mean values and standard deviations for the total sample and for subgroups defined by maternal genotype. In addition, table 3 shows the adjusted association between maternal passive smoking and infant birth weight. As estimated from the multiple linear regression model, passive smoking was not remarkably associated with a reduction in mean birth weight of 17.2 g (95 percent CI: −65.0, 30.6) in the total sample. There was also no significant association of passive smoking with infant birth weight with stratification by *CYP1A1 Msp*I genotype. However, this association differed by maternal genotype for *EPHX1 Tyr113His*. Passive smoking did not confer any adverse effect in the *Tyr/Tyr113* group but had a significantly negative effect in the *Tyr/His113* group (−103.5 g, 95 percent CI: −205.8, −9.2) and in the *His/His113* group (−687.3 g, 95 percent CI: −748.3, −178.3). In other words, for *EPHX1 Tyr113His*, the negative effects of maternal passive smoking on infant birth weight depended on maternal genotype.

We further assessed the association of amount of passive smoking with infant birth weight by considering passive smoking as a continuous variable (table 4). For every cigarette a mother was passively exposed to, mean birth weight decreased 18.0 g (95 percent CI: −30.2, −5.9) in the *CYP1A1 Msp*I *C/C6235* group and 10.4 g (95 percent CI: −19.8, −1.1) and 346.2 g (95 percent CI: −459.2, −15.2) in the *EPHX1 Tyr/His113* and *His/His113* groups, respectively.

Table 5 presents data on the combined association of passive smoking and maternal genotype with infant birth weight, where β represents the difference in mean birth weight between each subgroup and the reference group. In the absence of passive smoking, maternal genotype alone did not confer any significant adverse effect for either *CYP1A1 Msp*I or *EPHX1 Tyr113His*. However, in the presence of maternal passive smoking, remarkable reductions in mean birth weight of 112.1 g (95 percent CI: −179.3, −12.2) and 315.6 g (95 percent CI: −506.2, −87.1) were found in the *EPHX1 Tyr/His113* group and the *His/His113* group, respectively. A test of interaction between maternal passive smoking and maternal *EPHX1 Tyr113His* genotype was statistically significant for birth weight.

We also analyzed the combined association of passive smoking amount and maternal genotype with infant birth weight, as well as the association with infant birth weight when both *CYP1A1 Msp*I and *EPHX1 Tyr113His* were considered simultaneously in the presence or absence of passive smoking. However, there was no significant interaction (data not shown).

TABLE 4. Adjusted association of amount of passive smoking during pregnancy with infant birth weight, in total and by maternal cytochrome P-450 (*CYP1A1*) *Msp*I and epoxide hydrolase 1 (*EPHX1*) genotype,* Anqing, China, 1996–2000

Genotype	No. of participants	Mean birth weight (g)	Adjusted† β‡	95% confidence interval	*p* value
Total sample	1,388	3,107.6 (476.4)§	−1.8	−4.6, 1.0	0.213
*CYP1A1 Msp*I					
T/T6235	492	3,123.1 (495.9)	−1.9	−6.4, 2.7	0.416
T/C6235	768	3,099.8 (460.3)	0.1	−3.6, 3.9	0.946
C/C6235	128	3,095.3 (496.9)	−18.0	−30.2, −5.9	0.004
EPHX1					
Tyr/Tyr113	1,080	3,119.2 (488.0)	−1.6	−5.1, 0.3	0.294
Tyr/His113	276	3,070.3 (435.5)	−10.4	−19.8, −1.1	0.029
His/His113	32	3,040.0 (402.8)	−346.2	−459.2, −15.2	<0.001

* *Tyr/Tyr113* indicates the homozygous wild type; *Tyr/His113* indicates the heterozygous variant type; and *His/His113* indicates the homozygous variant type. *T/T6235* indicates the homozygous wild type; *T/C6235* indicates the heterozygous variant type; and *C/C6235* indicates the homozygous variant type.

† Results from a multiple linear regression model with adjustment for age (<25, 25–27, and >28 years), education (elementary school, middle school, and high school or above), shift work (no, yes), exposure to noise in the current job (no, yes), exposure to vibration in the current job (no, yes), perceived stress of the current job (no, yes), prepregnancy weight and height (linear and quadratic terms), cooking days, history of pregnancy (no, yes), and infant sex.

‡ β represents the difference in mean birth weight for each additional cigarette passively smoked by the mother after adjustment for the covariates listed above.

§ Numbers in parentheses, standard deviation.

DISCUSSION

Our study showed that the association of maternal *CYP1A1 Msp*I and *EPHX1 Tyr113His* genotypes with birth weight emerged only in those mothers who were passively exposed to environmental tobacco smoke. More importantly, the study provided consistent evidence that the adverse effects of maternal passive smoking on infant birth weight were modified by maternal *CYP1A1 Msp*I and *EPHX1 Tyr113His* genotypes. Our data demonstrated that a subgroup of pregnant women with certain genotypes appeared to be particularly susceptible to the adverse effect of passive cigarette smoking, suggesting an interaction between metabolic genes and passive smoking.

Although there are few published data on genetic susceptibility to maternal passive cigarette smoking in relation to birth weight, this susceptibility is biologically plausible. Persons exposed to environmental tobacco smoke are subjected to most of the same constituents as those contained in mainstream smoke, but the pattern and amounts of exposure differ (29). The potential passive smoke mechanisms are essentially the same as those for active smoking (12, 30), including vasocontriction and reduced placental blood flow due to nicotine (10), maternal and fetal hypoxia due to carboxyhemoglobin formation, and genotoxicity; that is, reduction of birth weight caused by fetal growth retardation may also be due to disturbance of cell regulation caused by DNA adducts and damage (31). There is evidence suggesting that toxicity is produced by one or more metabolites of cigarette smoke, particularly the covalent binding to cellular macromolecules, especially DNA (32, 33). DNA damage may result from some cytochrome P-450 variants (34) and the lack of detoxification of reactive tobacco smoke intermediates. Further studies (35, 36) demonstrated that levels of benzo(*a*)pyrene diol-epoxide–DNA adducts and bulky DNA adducts were significantly and positively correlated with CYP1A1 enzyme activity. Moreover, the microsomal form of epoxide hydrolase, EPHX 1, which is encoded by the *EPHX1* gene, catalyzes the conversion of a broad spectrum of highly reactive aliphatic epoxides to less toxic *trans*-dihydrodiols (18). Thus, a person's ability to convert toxic metabolites of cigarette smoke to less harmful moieties is important for minimizing the toxic effect on birth outcomes. *CYP1A1 Msp*I variant genotypes may increase enzyme activity (37), while the substitution of histidine for the more common tyrosine at codon 113 in exon 3 of *EPHX1* may lead to a decrease in enzyme activity (22). Consistently, our study found that the passively smoking mothers who had the *CYP1A1 Msp*I variant genotype *C/C6235* or the *EPHX1 Tyr113His* variant genotype *Tyr/His113* or *His/His113*, which reduces a person's ability to convert toxic metabolites of cigarette smoke to less harmful hydrophilic compounds, had infants with significantly lower birth weights than the reference groups. Furthermore, the greater the amount of passive smoking to which the pregnant women were exposed, the more remarkably birth weight was reduced in the *C/C6235* group of *CYP1A1 Msp*I and the *His/His113* group of *EPHX1*, suggesting a dose-response relation between passive smoking and infant birth weight.

TABLE 5. Combined associations of passive smoking during pregnancy and maternal cytochrome P-450 (*CYP1A1*) *Msp*I and epoxide hydrolase 1 (*EPHX1*) genotype with infant birth weight, Anqing, China, 1996–2000

Passive smoking	Maternal genotype*	Crude analysis		Adjusted† analysis		
		No. of participants	Mean birth weight (g)	β‡	95% confidence interval	*p* value
	CYP1A1 MspI					
No	*T/T6235*	224	3,120.7 (452.3)§	Reference		
No	*T/C6235*	404	3,123.1 (438.4)	3.8	−72.1, 75.3	0.926
No	*C/C6235*	52	3,134.6 (443)	−8.1	−145.3, 129.3	0.907
Yes	*T/T6235*	268	3,125.1 (530.5)	5.5	−76.1, 82.4	0.896
Yes	*T/C6235*	364	3,073.8 (482.7)	−15.6	−90.5, 61.2	0.703
Yes	*C/C6235*	76	3,068.4 (531.9)	−92.8	−215.4, 21.3	0.114
Interaction¶						
Crude				−41.1	−123.0, 40.9	0.326
Adjusted				−38.3	−112.8, 36.4	0.314
	EPHX1 Tyr113His					
No	*Tyr/Tyr113*	512	3,119 (455.7)	Reference		
No	*Tyr/His113*	152	3,129.2 (406.1)	−23.6	−113.7, 50.3	0.616
No	*His/His113*	16	3,200 (363.3)	1.8	−212.5, 245.2	0.898
Yes	*Tyr/Tyr113*	568	3,119.3 (515.7)	4.6	−45.7, 60.5	0.746
Yes	*Tyr/His113*	124	2,998.1 (460.6)	−112.1	−179.3, −12.2	0.022
Yes	*His/His113*	16	2,880 (385.6)	−315.6	−506.2, −87.1	0.006
Interaction¶						
Crude				−140.9	−245.0, −36.9	0.008
Adjusted				−109.7	−218.8, −12.4	0.024

* *Tyr/Tyr113* indicates the homozygous wild type; *Tyr/His113* indicates the heterozygous variant type; and *His/His113* indicates the homozygous variant type. *T/T6235* indicates the homozygous wild type; *T/C6235* indicates the heterozygous variant type; and *C/C6235* indicates the homozygous variant type.

† Results from a multiple linear regression model with adjustment for age (<25, 25–27, and ≥28 years), education (elementary school, middle school, and high school or above), shift work (no, yes), exposure to noise in the current job (no, yes), exposure to vibration in the current job (no, yes), perceived stress of the current job (no, yes), prepregnancy weight and height (linear and quadratic terms), cooking days, history of pregnancy (no, yes), and infant sex.

‡ β represents the difference in mean birth weight between each subgroup and the reference group after adjustment for the covariates listed above.

§ Numbers in parentheses, standard deviation.

¶ Test for interaction: *p* value for test of the null hypothesis; β = 0 in the multiple linear regression models for the product term, passive smoking × genotype.

Several methodological limitations should be considered when interpreting our results. Maternal passive smoking was based on self-reports rather than objective measurements and thus may have been subject to reporting bias and misclassification, which may have caused underreporting of smoking status and underestimation of the effect of passive smoking. Second, we adjusted for several variables, including demographic characteristics, occupational exposure, and reproductive history, in the regression analyses. However, we cannot exclude the possibility of confounding by uncontrolled or inadequately controlled risk factors. For example, we made no attempt to assess nutritional status and maternal weight gain during pregnancy, and socioeconomic status was not considered in our study. Third, the association between genetic susceptibility to passive smoking and reduced birth weight found in this study may be causal or may be a marker for other polymorphisms which are the true susceptibility loci or biologic pathways, because of the possibility of linkage disequilibrium. For example, the *EPHX1 His139Arg* polymorphism, substitution of arginine for histidine at codon 139 in exon 4, which increases EPHX 1's enzymatic activity by 25 percent, could modify the effect of the loss-of-function variant *Tyr113His* with respect to low birth weight (38). In addition, the *Ile462Val* polymorphism in exon 7 of *CYP1A1* is usually linked with *Msp*I and may increase the activity of aryl hydrocarbon hydroxylase, and is particularly relevant to the metabolism of chemicals in cigarette smoke. Fourth, there may be potential interactions with polymorphisms in genes for other phase II enzymes such as glutathione *S*-transferase T1 (*GSTT1*) and

glutathione *S*-transferase M1 (*GSTM1*), which are also reported to be associated with a lack of or reduction in enzymatic activity toward several substrates, including those found in tobacco smoke (39–42), and which may modify the effects of *CYP1A1 Msp*I and *EPHX1 Tyr113His* to some extent. Extensive efforts should be made to examine other polymorphisms to develop haplotype studies and to further test for these interactions. In addition, we only examined maternal genotypes, and the roles of fetal genotypes in modifying the adverse effects of passive smoking and maternal-fetal gene interaction remain to be determined.

In conclusion, we have demonstrated that the association between maternal passive smoking and reduced infant birth weight is significantly modified by maternal genotype. This study provides evidence of gene-environment interaction and suggests the importance of further assessing the role of genetic susceptibility in the evaluation of reproductive toxins. A coherent gene-environment interaction approach may help to identify high-risk subpopulations for clinical or public health interventions.

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CHAPTER 3

Genetic and environmental influences on objective intermediate asthma phenotypes in Dutch twins

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Genetic and environmental influences on objective intermediate asthma phenotypes in Dutch twins

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ABSTRACT: It is unclear to what extent the same set of environmental or genetic factors regulate objective intermediate asthma phenotypes. We examined heritabilities of these phenotypes and estimated their environmental and genetic overlap.

We studied baseline lung function (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC), bronchial hyperresponsiveness, number of positive skin prick tests (SPT) to 11 allergens, serum total immunoglobulin (Ig)E, number of positive specific IgE tests to four allergens and eosinophil counts. 103 twin pairs were studied (46 monozygotic and 57 dizygotic; mean age: 22.5 yrs, range: 17.0–27.0 yrs). Univariate and bivariate genetic analyses were performed after adjustment for significant covariates.

All intermediate asthma phenotypes showed significant heritabilities (47–83%). Most phenotypes were substantially correlated, which was mainly due to shared genetic factors. Pairs of phenotypes with the largest genetic correlations were specific IgE and SPT (0.98), and total IgE with specific IgE (0.87), with SPT (0.72), and with eosinophils (0.62). SPT showed significant environmental correlations with total IgE (0.65), specific IgE (0.70) and bronchial hyperresponsiveness (0.44).

Genetic effects explain the majority of the variation in objective intermediate asthma phenotypes. Additionally, correlations between pairs of these traits are also mainly explained by genetic rather than environmental factors.

KEYWORDS: Asthma, environmental correlation, genetic correlation, multivariate, twin

Asthma afflicts millions of people worldwide and is caused by multiple genetic and environmental factors. There has been a strong interest in searching for susceptibility genes for asthma, driven by the prospect of better disease prevention, diagnosis and treatment. However, studies on the genetics of asthma are complicated, since there are difficulties in standardising the diagnosis of asthma [1, 2]. Most current genetic studies have concentrated on asthma associated with atopy, defined in a number of different ways: by elevated total serum immunoglobulin (Ig)E levels and/or positive skin prick tests (SPT) to one or more allergens. The binding of >IgE to its receptors results in mast cell activation and eosinophil recruitment, another asthma-related phenotype. Bronchial hyperresponsiveness, the increased bronchoconstrictor response to nonallergic stimuli is a pre-requisite for an asthma diagnosis [3]. These measurable and biological markers, so-called intermediate phenotypes, such as IgE levels, SPT, eosinophils and bronchial hyperresponsiveness, underlie the pathophysiology and clinical expression of asthma [4]. They are more objective, accurate, and more informative to use in genetic analyses than clinical definitions or doctor's diagnosis of asthma. So far, only how far these intermediate phenotypes are driven by similar or different genetic or environmental factors has been studied.

Twin and family studies are widely used to estimate genetic and environmental contributions to atopic disease [5, 6]. The multivariate classical twin design can help to estimate the degree to which the same genetic and environmental factors influence different intermediate phenotypes [7]. If there is overlap in genes for two traits

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it is expected that the cross-twin cross-trait correlation will be higher in monozygotic (MZ) twins than in dizygotic (DZ) twins for these traits. Using this information cannot only widen our understanding of atopic comorbidity, it can also enhance gene-mapping efforts [6] and consequently enable us to understand the interplay between different pathways underlying asthma, benefitting the discovery of targeted treatments and interventional measures.

The aims of the current study were to estimate the relative influence of genetic and environmental factors on objective intermediate asthma phenotypes and, more importantly, to what extent correlations between these intermediate phenotypes can be explained by genetic and/or environmental factors using a sample of 103 young Dutch twin pairs.

METHODS

Subjects

The families participating in the asthma study were a sample of a larger number of Dutch twin families who participated in the Netherlands Twin Register (NTR), an ongoing survey study of health-related behaviour [8–10]. The NTR represents a random sample of Dutch families with twins, recruited from city council registrations. We selected families in which twins were aged ⩾18 yrs and who reported at least one member with a history of asthma. In total, 102 families participated, contributing 103 twin pairs (one family contributed two twin pairs) of which 46 pairs were MZ and 57 pairs were DZ, of which 26 pairs were of opposite sex (for details see the supplementary material). Zygosity was determined by DNA fingerprinting (Sequana, San Diego, CA, USA and TNO, Leiden, The Netherlands). The study was approved by the institutional review board and all subjects provided written informed consent.

Measurements

We used the European Community Respiratory Health Survey questionnaire to define the symptoms presented in table 1 (*e.g.* asthma, cough, wheeze and dyspnoea, *etc.*).

Lung function test

Lung function was measured using pneumotachographs (Vmax series, Sensor Medics Co., Yorba Linda, CA, USA) according to the guidelines of the American Thoracic Society [11]. Each twin pair was tested on the same day and at the same time of day. Forced vital capacity (FVC) measurement was followed by measuring of the forced expiratory volume in 1 s (FEV_1). Three measurements were made until at least two satisfactory measurements were produced; the highest values being taken as baseline value. The ratio of FEV_1 and FVC was used to determine the degree of airway obstruction.

Bronchial hyperresponsiveness

Bronchial hyperresponsiveness (BHR) was measured on a DeVilbiss 646 nebuliser (Devilbiss Co., Somerset, PA, USA) using the standardised protocol described by COCKCROFT *et al.* [12], based on 2-min inhalations of doubling concentrations of methacholine of 0.03–160 mg·mL-1 (see the supplementary material). The methacholine provocation concentration producing a 20% fall in FEV_1 (PC_{20}) was determined using linear interpolation of the last two points of the concentration-response curve [13].

Skin prick test

Skin prick tests were performed using allergen-coated lancets for 11 common allergens: house dust mite, storage mite, mixed tree pollens, mixed grass, weed, cat, dog, horse, hair, feathers and moulds. A participant was defined as atopic if at least one of these allergens tested elicited a mean wheal diameter ⩾3 mm [14] and the negative control had a mean wheal diameter <1 mm.

Measurements of total serum IgE, specific IgE and eosinophil counts

Measurements methods of serum total IgE and specific IgE are described in the supplementary material. Peripheral blood eosinophil counts were performed by flow cytometry.

Analytical approach

The aims of our analyses were two-fold. First, we estimated the relative influence of genetic and environmental factors on observed phenotypic variance in eight objective intermediate asthma phenotypes separately and investigated sex differences in genetic architecture. Secondly, we assessed to what extent phenotypic correlations between these phenotypes can be explained by genetic and/or environmental factors. We used bivariate modelling to estimate cross-trait correlations and to partition these into genetic and environmental components.

Twin correlations and heritability estimates

Most phenotypes were adjusted for covariates prior to the estimation of twin correlations and model fitting. FEV_1, FVC and FEV_1/FVC were adjusted for age, sex, height and smoking. PC_{20} was adjusted for age and sex. Total IgE and eosinophil counts were log-transformed to obtain normal distribution and both were adjusted for age and sex.

Pearson correlations were calculated for continuous traits. Polychoric correlations were calculated for ordinal traits (*i.e.* PC_{20}, SPT and specific IgE) under a liability-threshold model [7, 15] (for details see the supplementary material). Although PC_{20} was measured as a continuous variable, it could not be normalised in our study. Therefore, we recoded PC_{20} into an ordinal variable with six categories. Lower recoded values represent higher actual values (*e.g.* 0 represented 160 $mg \cdot ml.^{-1}$), indicating a lower bronchial responsiveness. SPT and specific IgE were also defined as ordinal variables with six and four categories, respectively, based on the total number of positive responses. Heritabilities for all traits were estimated using univariate genetic model fitting techniques.

Genetic modelling of twin data

We used model fitting analyses of twin data [16], a technique based on comparison of covariances (or correlations) in MZ and DZ twin pairs, which allowed a separation of the observed phenotypic variance into its genetic and environmental components (fig. 1): additive or dominant genetic components and common or unique environmental components. The unique environmental component also contains measurement error. Dividing each of these components by the total variance yields the different standardised components of variance, for example, the heritability (h^2) can be defined as the proportion of the total variance attributable to additive genetic variation.

TABLE 1 General characteristics and objective intermediate asthma phenotypes of male and female twins

Characteristics	Males	Females	Sex effects p-value
Subjects n	90	116	
Age yrs	22.4±1.6	22.7±1.9	NS
Height m	1.82±0.07	1.68±0.07	<0.001
Weight kg	73.3±9.9	61.8±9.4	<0.001
BMI kg·m^{-2}	22.0±2.6	21.8±2.9	NS
Current smoking	26 (28.9)	27 (23.3)	NS
Asthma	47 (52.2)	61 (52.6)	NS
Cough	17 (18.9)	36 (31.0)	NS
Wheeze	24 (26.7)	38 (32.8)	NS
Dyspnoea	28 (31.1)	46 (39.7)	NS
Dyspnoea at night	5 (5.6)	24 (20.7)	0.001
Medication			
β agonists	7 (7.8)	20 (17.2)	<0.05
Inhaled corticosteroid	2 (2.2)	11 (9.5)	<0.05
Anticholinergic	1 (1.1)	1 (0.9)	NS
Objective intermediate asthma phenotypes#			
FEV_1 L	4.7±0.7	3.4±0.4	<0.001
FEV_1 % pred	98.6±20.5	91.3±22.4	NS
FVC L	5.6±0.7	4.0±0.6	<0.001
FVC % pred	96.3±18.9	95.4±22.5	NS
FEV_1/FVC	0.83±0.09	0.86±0.08	<0.05
Total serum IgE IU·mL^{-1}	86.5 (24.5–202)	53 (21–172)	NS
Eosinophil count × 10^9 cells·L^{-1}	0.14 (0.09–0.22)	0.11 (0.07–0.21)	NS
Methacholine PC_{20} mg·mL^{-1}	139.5 (18.45–160)	41.8 (4–160)	<0.05
Positive SPT	30 (33.3)	49 (42.2)	NS
Positive serum specific IgE	45 (51.1)	51 (47.2)	NS

Data are presented as mean±SD, n (%) or median (interquartile range), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; % pred: % predicted, corrected for age and height; FVC: forced vital capacity; Ig: immunoglobulin; PC_{20}: provocative concentration causing a 20% fall in FEV_1; SPT: skin prick test; NS: not significant. #: missing value for these phenotypes varied from 0 (0%, FVC and SPT) to 13 (6.31%, PC_{20}).

The existence of sex differences in the influences of genetic and environmental factors on the phenotype can take several forms [16] (see the supplementary material). A path diagram of the applied twin model is shown in figure 1; k is the scalar factor that indicates that the total variance of the phenotype might differ between males and females.

Bivariate genetic models

In order to estimate genetic and environmental influences on the association between these intermediate asthma phenotypes, a bivariate Cholesky decomposition [16] was used to model the covariance between any two of them (fig. 2). This model allows determination of the extent to which the covariance (or phenotypic correlation (rp)) can be explained by genetic and/or environmental factors influencing both phenotypes under study (see the supplementary material). The genetic correlation (rg) between two traits gives an indication of the amount of overlap between (sets of) genes influencing those traits and ranges from -1 to 1. If rg is 1, then the same genes influence both traits. A correlation of 0 indicates that both traits are affected by a different set of genes. The positive or negative genetic correlation reflects positive or negative association between any two traits. Common and unique environmental correlations between two traits are calculated in a similar way (fig. 2). In order to include both continuous and ordinal (PC_{20}, SPT and specific IgE) variables into the same bivariate model, we recoded continuous variables to ordinal ones with 10 categories.

Model fitting procedure

Models were fitted to the raw data using normal theory maximum likelihood allowing inclusion of incomplete data (*i.e.* when data were only available in one twin of a pair). Depending on the correlation pattern for MZ and the DZ twins, the genetic model fitting started with either an ACE (additive genetic, common environmental, unique environmental) model (if twice the DZ correlation was larger than the MZ correlation) or an ADE (additive genetic, dominance genetic, unique environmental) model (if the DZ correlation was less than half the MZ correlation). The significance of variance components, additive genetic, common environmental or dominance genetic, was assessed by testing the deterioration in model fit after each component was dropped from the full model. Standard hierarchic Chi-squared tests were used to select the best fitting models [16] in combination with Akaike's information criterion (AIC = χ2-2 degrees of freedom). The model with the lowest AIC reflects the best balance of goodness-of-fit and parsimony.

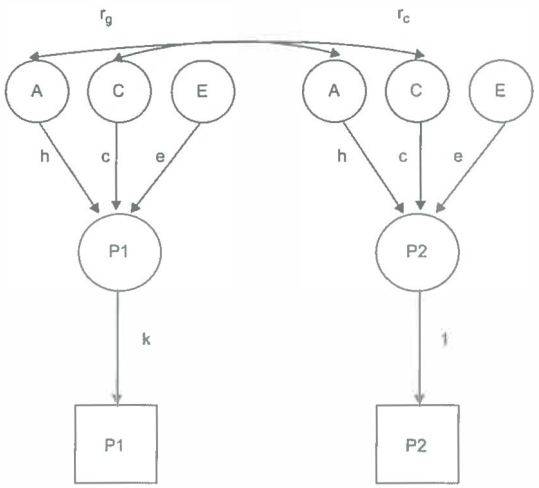

FIGURE 1. Path diagram for a univariate model. An opposite-sex twin pair is shown, twin 1 (left) being male and twin 2 (right) female. Observed phenotypes (P) for twin 1 and twin 2 are shown in squares, latent (i.e. unmeasured) factors are shown in circles. A: additive genetic factor; C: common environmental factor; E: unique environmental factor. r_g is the correlation between A, which is 1 in monozygotic (MZ) twins and 0.5 in dizygotic (DZ) same sex twins, while r_c is the correlation between C, which is 1 in MZ twins and DZ same sex twins. r_g and r_c can be estimated for DZ opposite sex twins. Regression coefficients of observed variables on the different latent factors are shown in lower case: h: additive genetic effect; c: common environmental effect; e: unique environmental effect; k: scalar factor. Heritabilities are constrained to be equal across sexes in this model, but total variances may be different. All (nonstandardised) variance components for females were constrained to be equal to a scalar multiple, k^2, of the male variance components. Dominance genetic influence was also tested but is omitted to simplify the diagram.

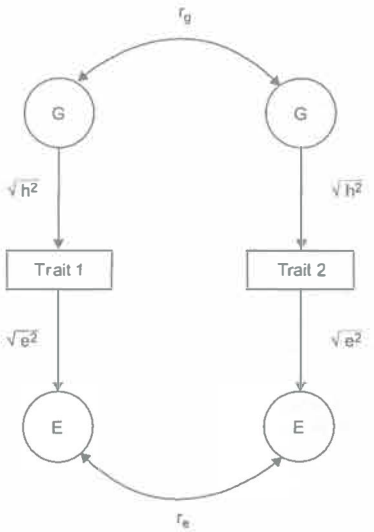

FIGURE 2. Genetic and environmental correlations and factor loadings of the best fitting bivariate model for any two asthma-related traits. For clarity, only one twin is depicted. Factor loadings (or path coefficients) are expressed as square roots to make clear that squaring of those factor loadings yields estimates of genetic and environmental variance components, as shown in the text. G: additive genetic factor; h^2: heritability; e^2: unique environmental variance component; E: unique environmental factor; r_g: genetic correlation; r_e: environmental correlation.

Statistical software

Effects of sex and other covariates on mean values were tested by generalised estimating equations (GEE). GEEs take the non-independence between twins into account and yields unbiased standard errors and p-values [17]. Data handling, preliminary analyses, and GEEs were performed with STATA software (StataCorp., College Station, TX, USA). Polychoric correlations were calculated and genetic modelling performed with Mx software (Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA, USA), a computer program specifically designed for the analysis of twin and family data [18].

RESULTS

Table 1 shows the characteristics of the male and female twins. The mean age of the population was 22.5 yrs (range 17.0–27.0 yrs). The total number of asthmatics was 108 individuals. As shown in table 1, age, body mass index and current smoking status were comparable in males and females. Males were taller and heavier than females and showed significantly higher FEV_1, FVC and PC_{20} values but lower FEV_1/FVC values compared with females. Females reported more often to have dyspnoea at night and used medication more often than males. None of the traits showed significant differences between MZ and DZ twins.

Table 2 presents the twin correlations of the objective intermediate asthma phenotypes by zygosity groups. Twin correlations were collapsed across sex, because heritability estimates were not significantly different between males and females (see below and table 3). MZ correlations were consistently higher than DZ correlations, indicating an important contribution of genetic factors. Parameter estimates and 95% CIs of these best-fitting models are presented in table 3. All univariate models of the eight objective asthma-related phenotypes showed significant heritabilities (h^2 range: 47–83%). A significant scalar sex effect (fig. 1) was found for FEV_1, with males showing larger variability than females.

Subsequently, we performed bivariate model fitting to estimate cross-trait correlations and investigate to what extent these correlations can be explained by shared genetic or shared environmental factors influencing both phenotypes (table 4 and fig. 3). There were negative cross-trait correlations between lung function variables and all other traits. That is, atopy and BHR were related to lower lung function levels. Phenotypic correlations ranged 0.18–0.86 (irrespective of the sign). The strongest correlations were among serum total IgE, specific IgE and SPT (range 0.68–0.86). There were nonsignificant correlations of eosinophil counts with FEV_1 (-0.13) and FEV_1/FVC (-0.12), and of SPT with FEV_1/FVC (-0.16).

TABLE 2 Twin correlations by zygosity of objective intermediate asthma phenotypes

Measures	MZ	DZ
Pairs n	46	57
FEV1	0.77	0.27
FVC	0.72	0.36
FEV1/FVC	0.64	0.32
Total serum IgE	0.73	0.43
Serum specific IgE	0.60	0.29
Eosinophils	0.54	0.28
Methacholine PC20	0.50	0.16
SPT	0.57	0.25

MZ: monozygotic; DZ: dizygotic; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; Ig: immunoglobulin; PC20: provocative concentration causing a 20% fall in FEV1; SPT: skin prick test. FEV1, FVC and FEV1/FVC were adjusted for age, sex, height and smoking; total IgE, eosinophils and PC20 were adjusted for age and sex. Pearson correlations were used for continuous traits (FEV1, FVC, FEV1/FVC, total IgE and eosinophils) and polychoric correlations were used for the ordinal variables (PC20, specific IgE and SPT). Recoding of PC20: 0=5, 0=160, larger values indicating higher bronchial responsiveness. Coding of SPT: 0–5, larger values indicating more positive responses to the allergens. Coding of specific IgE: 0–3, larger values indicating more positive responses to the allergens.

TABLE 3 Parameter estimates and 95% CIs of best-fitting models for univariate analyses of objective intermediate asthma phenotypes

Phenotype	Variance components	
	h^2 (95%CI)	e^2 (95%CI)
FEV1[#]	0.83 (0.70–0.89)	0.17 (0.11–0.30)
FVC	0.72 (0.56–0.82)	0.28 (0.18–0.44)
FEV1/FVC	0.61 (0.43–0.74)	0.39 (0.26–0.57)
Total serum IgE	0.75 (0.60–0.84)	0.25 (0.16–0.40)
Serum specific IgE	0.60 (0.31–0.80)	0.40 (0.20–0.70)
Eosinophils	0.52 (0.29–0.69)	0.48 (0.31–0.71)
PC20	0.47 (0.17–0.70)	0.53 (0.30–0.83)
SPT	0.56 (0.27–0.77)	0.44 (0.23–0.73)

h^2: heritability; e^2: unique environmental variance component; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; Ig: immunoglobulin; PC20: provocative concentration causing a 20% fall in FEV1; SPT: skin prick test. FEV1, FVC and FEV1/FVC were adjusted for age, sex, height and smoking; total IgE, eosinophils and PC20 were adjusted for age and sex. PC20, SPT and specific IgE were analysed as ordinal variables with a liability model (five thresholds for PC20 and SPT, three thresholds for specific IgE). [#]: scalar sex effect, male>female (k=1.52).

Serum total IgE showed significant genetic correlations with all other traits (range -0.32–0.87). Specific IgE also presented high genetic correlations with most traits (range -0.46–0.98) and especially a very high correlation with SPT (0.98). Similarly, substantial genetic correlations of FEV1 were observed with FEV1/FVC (0.52), PC20 (-0.51), specific IgE (-0.46) and SPT (-0.43). By contrast, eosinophil counts showed significant genetic correlations with total IgE (0.62) and specific IgE (0.50) only.

Compared with genetic effects, fewer environmental correlations were significant. FEV1 and FEV1/FVC were environmentally correlated (0.43), as were FEV1/FVC and total IgE (-0.31), and SPT and PC20 (0.44). Total IgE, specific IgE and SPT showed considerable environmental overlap with each other (range 0.65–0.77).

Figure 3 presents the results that decompose the phenotypic correlation into common genetic and environmental factors. These results confirm the expectation that much of the phenotypic correlations between these objective asthma-related traits are attributable to genetic factors, except for the correlation between PC20 and SPT, which is mainly attributable to environmental factors.

DISCUSSION

The aim of this study was to estimate the relative influence of genetic and environmental factors on individual differences in eight objective intermediate asthma phenotypes including baseline lung function (FEV1, FVC and FEV1/FVC), BHR, positive SPT, serum total and specific IgE, and eosinophil counts of importance, we investigated environmental and genetic overlap between these phenotypes in a subsample of young twins from the NTR. In addition to the findings of genetic effects accounting for a substantial portion (≥47%) of the variation of all asthma phenotypes, the main finding of our study was that these objective intermediate asthma phenotypes had significant cross-trait correlations, which were predominantly explained by genetic rather than by environmental factors.

We observed heritabilities of 61–83% for lung function phenotypes; higher than in previous reports [19, 20]. This may be due to the somewhat younger age of our cohort of twins, in which environmental factors, such as smoke exposure may have had less influence. The current study is one of the few focusing on comprehensive clinical markers of allergy, thereby showing that the heritability of specific IgE is 0.60 in twins when analysing it as ordinal variable based on the sum score of the positive responses to four allergens. Few twin studies have included SPT [19, 21] and heritability estimates were not consistent (0.49 *versus* 0.85). We also analysed SPT as an ordinal variable and found a heritability of 0.56. We did not find any sex effect on heritability of these traits, except for a significant scalar effect with FEV1, males showing larger variability than females.

We did not find any evidence for environmental factors contributing to intermediate phenotypes that are shared among twins of a pair, probably due to gene–environment interactions. It is likely that environmental risk factors trigger asthma only in subjects with a larger genetic susceptibility for allergic and asthmatic diseases. Evidence for linkage between certain chromosomes and asthma were, for example, only found in those who where exposed to cigarette smoke in early childhood; in other words, certain genes may be expressed only upon environmental exposure [22–24]. These results emphasise the importance of considering environmental information in genetic studies of asthma and its related traits.

TABLE 4 Genetic (below main diagonal) and environmental (above main diagonal) correlations between objective intermediate asthma phenotypes

	FEV_1	FEV_1/FVC	Total serum IgE	Serum specific IgE	Eosinophils	PC_{20}	SPT
FEV_1		**0.43**	0.19	0.18	-0.05	-0.25	0.17
FEV_1/FVC	**0.52**		**-0.31**	-0.32	-0.06	-0.23	0.13
Total serum IgE	**-0.30**	**-0.32**		**0.77**	0.15	0.18	**0.65**
Serum specific IgE	**-0.46**	-0.32	**0.87**		0.25	0.20	**0.70**
Eosinophils	-0.18	0.18	**0.62**	**0.50**		0.25	0.21
PC_{20}	**-0.51**	0.24	**0.42**	**0.57**	0.12		**0.44**
SPT	**-0.43**	-0.21	**0.72**	**0.98**	0.36	0.23	

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; Ig: immunoglobulin; PC_{20}: provocative concentration causing a 20% fall in FEV_1; SPT: skin prick test. FEV_1, FVC and FEV_1/FVC were adjusted for age, sex, height and smoking; total IgE, eosinophils and PC_{20} were adjusted for age and sex. PC_{20}, SPT and specific IgE were analysed as ordinal variables with a liability model (five thresholds for PC_{20} and SPT; three thresholds for specific IgE). Significant correlations are shown in bold.

A specific focus of this paper was to explore both genetic and environmental correlations between objective intermediate asthma phenotypes by performing bivariate variance components analyses. In this way, all traits associated with allergy, *e.g.* BHR, eosinophils, total and specific IgE, and SPT, presented significant cross-trait correlations. Thus, our findings support the pathophysiological connection arising from shared genetic determinants. The genetic correlation between eosinophils and total IgE (0.62) that we found fits with the finding of genetic linkage of eosinophilia to 2q33 [25], a region previously reported to be linked with serum total IgE [26], and is also in accordance with previous findings [27] that 2q24–32 showed evidence of linkage for both eosinophils and total serum IgE in Dutch families. Furthermore, the latter study showed considerable genetic overlap among total IgE, specific IgE and SPT. Specific IgE and SPT were linked to chromosome 17q25 and 22q11, and total IgE and specific IgE to chromosome 7q11–q12 [27]. Interestingly, there were comparatively strong genetic overlaps of FEV_1 with PC_{20} and SPT as well as FEV_1/FVC. These results are in accordance with previous findings, which have reported that FEV_1, atopy and BHR were all linked to chromosome 20q13. This linkage may result from a quantitative

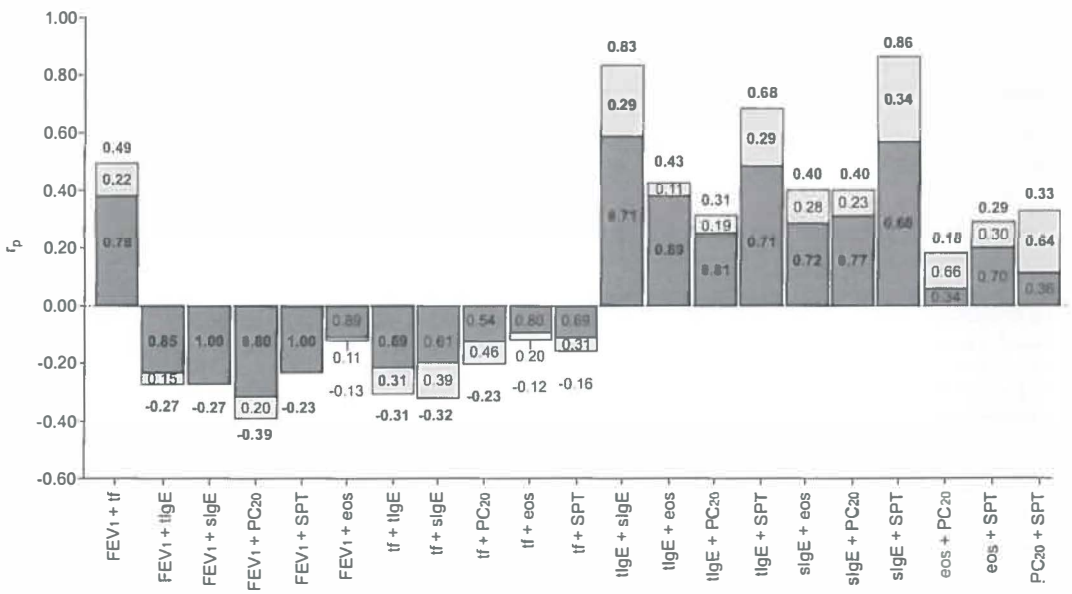

FIGURE 3. Bivariate analyses for objective intermediate asthma phenotypes. The height of the bars in the figure corresponds to the magnitude of the phenotypic correlations (r_p). The bars are partitioned into components reflecting proportions of r_p that are accounted for by genetic (■) and environmental (□) factors. FEV_1: forced expiratory volume in 1 s; Ig: immunoglobulin; PC_{20}: provocative concentration causing a 20% fall in FEV_1; SPT: skin prick test; FVC: forced vital capcity; tf: FEV_1/FVC; tIgE: serum total IgE; sIgE: specific IgE; eos: eosinophils. Significant correlations are shown in bold.

trait locus in this region that affects several asthma-related traits [28].

Bivariate analyses also showed that a smaller number of phenotypes had significant environmental correlations. In accordance with findings of FERREIRA *et al.* [19], we found that there were environmental correlations of SPT with both specific IgE and PC_{20}, indicating that exposure and sensitisation to aeroallergens are fundamental for development of both asthma and rhinitis. This is plausible, given the observation that inhalation of allergens to which an individual is allergic induces a more severe hyperresponsiveness, or even renders an individual hyperresponsive during the allergic season [29–31]. However, the fact that unique genetic and environmental effects were also present for any pairs of traits (*i.e.* genetic or environmental correlation were not equal to 1) indicates that they are still distinct intermediate phenotypes, even though their genetic or environmental aetiology is partly the same.

A strength of our study is that we have analysed noncontinuous traits (specific IgE, PC_{20} and SPT) as ordinal variables (more than two categories) using a threshold model [7, 32], whereas similar previous studies merely analysed these as binary (yes/no) traits. It is known that threshold models of ordinal traits are more powerful for detecting additive genetic or common environmental factors [33].

Finally, some limitations need to be considered. First, the ratio of MZ pairs to DZ pairs (46:57) was higher than in the general population [34]. However, oversampling of MZ twins was done on purpose to achieve similar group sizes of MZ and DZ twins, as is commonly applied in volunteer twin studies. Apart from a slightly lower power to detect common environmental factors [34], this is unlikely to have influenced results or generalisability of the study. Secondly, selective ascertainment of families was present in our study. Subjects studied only came from families with twins aged $\geq$18 yrs with at least one member (twins or their parents) reported a history of asthma, leaving us with a fraction of the original sample. Although this potentially may have impact on the estimates, a previous study comparing the heritability estimates in selected samples with those in the entire original sample [35], indicated that using the entire or selected sample gave very similar heritabilities.

In conclusion, genetic effects account for a substantial part of the variation in all objective intermediate asthma phenotypes. The correlations between pairs of these traits are, to a large extent, explained by genetic effects influencing both phenotypes. Environmental factors also contributed to the clinical homogeneity between asthma traits, but to a smaller extent. These findings enlarge our knowledge on the genetic and environmental origins of clinical homogeneity for these objective asthma phenotypes, and provide important clues and directions for gene-finding studies.

STATEMENT OF INTEREST

None declared.

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CHAPTER 4

Urinary norepinephrine and epinephrine excretion rates are heritable, but not associated with office and ambulatory blood pressure

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ORIGINAL ARTICLE

Urinary norepinephrine and epinephrine excretion rates are heritable, but not associated with office and ambulatory blood pressure

Fokko J Bosker[1,5], Ting Wu[2,5], Anatoliy Gladkevich[1], Dongliang Ge[3], Frank A Treiber[4] and Harold Snieder[2,3]

Genetic and environmental contributions to urinary excretion rates of norepinephrine ($U_{NE}V$) and epinephrine (U_EV) and their association with blood pressure (BP) were investigated in 91 African American (mean age, 17.3 ± 2.6 years) and 101 European American (mean age, 18.7 ± 3.4 years) mono- and di-zygotic twins. Genetic modeling was performed using Mx software. $U_{NE}V$ (1.9 ± 1.3 μg h^{-1}) and U_EV (0.2 ± 0.2 μg h^{-1}) were highly correlated ($r = 0.81$, $P < 0.001$). Significant heritabilities for $U_{NE}V$ (0.68) and U_EV (0.74) without ethnic and gender effects were observed. The genetic correlation between $U_{NE}V$ and U_EV was 0.86. There was no clear pattern of correlations for $U_{NE}V$ and U_EV with BP measures in European Americans, but African Americans showed some inverse correlations of moderate size. Measurements of $U_{NE}V$ and U_EV provide a viable method for the study of sympathetic tone and are substantially heritable.
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Keywords: blood pressure; epinephrine; heritability; norepinephrine; twin

INTRODUCTION

The catecholamines norepinephrine (NE) and epinephrine (E) mediate the early stress response via the sympathetic nervous system,[1,2] but they also have a key role in homeostatic blood pressure (BP) control[3,4] through the activation of adrenergic receptors located on the heart and the blood vessels. It has been suggested that abnormally increased sympathetic function may lead to the development and progression of a hypertensive state[3,5] perhaps initially by influencing transient BP increases to environmental stress.[6,7] Indeed, several studies indicate that this so-called sympathetic overdrive is a hallmark of essential hypertension[8,9] and several mechanisms and consequences have been proposed, with particular emphasis on its role in the development of target organ damage.[10] The substantial dysregulation of sympathetic function in patients with essential hypertension, as well as in their normotensive offspring, has incited research into susceptibility genes and chromosome loci being associated or linked with sympathetic function and essential hypertension.[11]

As such, identification of genes that influence catecholamine levels (in blood) or excretion rates (in urine) as proxies for sympathetic nervous system activity may improve our insight into the potential role of the sympathetic system in the early stages of essential hypertension. A prerequisite of such gene-finding studies is that heritability of the trait of interest is firmly established. However, very few studies investigated heritability of NE and E levels or excretion rates and none of these examined to what extent genetic and environmental influences on these catecholamines overlap.[5,6,12–14]

In the present study, we have used overnight urinary excretion rates of NE ($U_{NE}V$) and E (U_EV) as measures of basal sympathetic activity. The overnight urine collections were analyzed by radio immune assay (RIA) for NE and E. We aimed to determine the genetic and environmental contributions to $U_{NE}V$ and U_EV and their association using bivariate genetic modeling, and examine the associations of $U_{NE}V$ and U_EV with office and ambulatory BP in 91 African-American and 101 European-American adolescent and young adult twins from the south-eastern USA.

METHODS

Subjects

The present study comprised subjects from the Georgia Cardiovascular Twin Study.[15–17] After excluding 28 individuals who did not have overnight urine volume data, which was used to estimate $U_{NE}V$ and U_EV, participants were 101 European-American and 91 African-American twins (84 pairs and 24 individuals) including monozygotic (MZ) pairs and dizygotic (DZ) pairs

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of the same and the opposite sex (mean age: 18.0 ± 3.1 years, range: 12.0 to 29.2 years). The Medical College of Georgia Institutional Review Board approved the protocol. Written informed parental and subject consents were obtained from each participant family. Zygosity determination and recruitment have been described previously,[17–19] as have been the criteria to classify subjects as European- or African-American.[20] All of the subjects were apparently healthy, based on parental report of the children's medical history. None of the subject used any antihypertensive medication.

Protocol

Eligible twins (that is, healthy with no chronic illness, not on any prescribed medication including contraceptives and not pregnant) were sent instructions on how to collect and bring an overnight urine sample at the start of the testing day, which was used to determine $U_{NE}V$ and U_EV in the current study.[21] Twins recorded the last time they voided before retiring to bed and the time of their morning void, which they brought into the laboratory. The mean (s.d.) collection time for these overnight samples in our study was 7.64 (1.71) h. Twins arrived at the laboratory in the morning between 8:00 and 9:00 AM in pairs. The subjects (and their parents if the twins are < 18) were instructed to refrain from consuming foods or beverages (except water), tobacco and alcohol for 11 h before the visit (that is, fasting state) and to refrain from taking non-prescription medications for 2 days before the visit. A minority of twin pairs was scheduled for afternoon visits. These twins were told to refrain from consuming food and beverages (except water), tobacco and alcohol for 5 h before their visit.

Anthropometric measures

Anthropometric measurements were obtained using previously established protocols.[22] Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Body surface area (BSA) was calculated according to the Mosteller formula[23] as the square root of [height (cm) × weight (kg)/3600].

$U_{NE}V$ and U_EV measures

RIA kits (ALPCO, Salem, NH) were used to determine overnight urine concentrations of NE and E. The average intra-assay coefficients of variation for this kit are 4.3 and 9.3% and the inter-assay coefficients of variation are 8.1 and 5.9% for NE and E, respectively. For NE, we tested the performance of the RIA in 10 samples across a wide range of values against the high-performance liquid chromatography method of analysis and found virtually identical results ($r = 0.994$) (Figure 1). Excretion rates of NE ($U_{NE}V$) and E (U_EV) (in μg/h) were calculated as: (concentration × overnight volume)/overnight collection duration.

Office BP recordings

Office systolic BP (SBP) and diastolic BP (DBP) were measured with the Dinamap Vital Signs Monitor (model 1864 SX; Criticon Incorporated, Tampa, FL, USA). BP measurements were taken at the 11th, 13th and 15th minutes during a 15-minute supine relaxation period. The average of the last two readings was used to represent office SBP and DBP values.[20]

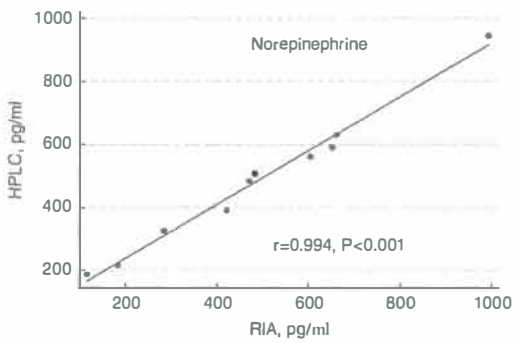

Figure 1 Association of NE concentration determined by RIA and high-performance liquid chromatography ($r = 0.994$).

Ambulatory BP recordings

Our procedures for ambulatory BP recordings have previously been described in detail.[24,25] Briefly, an ambulatory BP monitor was fitted to the non-dominant arm (model 90207, SpaceLabs, Redmond, WA, USA). Measures were obtained every 20 min during the daytime (08:00 to 22:00 h), and every 30 min during the night time (00:00 to 06:00 h). Transitional periods from 06:00 to 08:00 h and 22:00 h to midnight were not included in daytime and night time period. Adequacy of recordings was based on acceptable readings using previously established criteria[24] for ⩾14 readings over the 14 h designated as daytime and ⩾6 readings over the 6h designated as the night time, as suggested by the European Society of Hypertension Working Group on Blood Pressure Monitoring.[26] For the calculation of 24-h mean values (for which transition periods were included), 1-h mean values were first calculated. Subsequently these 1-h mean values were averaged.

Statistical analysis

The major aims of our study were threefold. First, we tested the association of $U_{NE}V$ and U_EV with office and ambulatory BP using correlational analyses. Second, we used univariate model fitting analyses to estimate the relative influence of genetic and environmental factors on individual differences in $U_{NE}V$ and U_EV and investigated gender and ethnicity differences in those variance components. Third, we used a bivariate model including both $U_{NE}V$ and U_EV to estimate the following: (1) the extent to which the phenotypic correlation between $U_{NE}V$ and U_EV can be explained by genetic and/or environmental factors influencing both traits; (2) the extent to which genetic and environmental effects on $U_{NE}V$ are the same or different from those affecting U_EV.

Correlational analyses. Before testing the association of $U_{NE}V$ and U_EV with office and ambulatory BP $U_{NE}V$ and U_EV were log-transformed and adjusted for BSA. BP measures (office SBP/DBP, 24 h SBP/DBP, night time SBP/DBP and daytime SBP/DBP) were adjusted for age and BMI. For both European and African Americans, correlations were calculated overall and in males and females separately. The non-independence between twins was taken into account in calculating the significance of the correlations.

Univariate modeling of twin data. Structural equation modeling was the primary method of analysis. Structural equation modeling is based on the comparison of the variance–covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into its genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components.[27] We used the ADE model as our initial full model, if correlations among MZ twins substantially exceeded twice those among DZ twins, which would indicate dominance variance; otherwise we used the ACE model.[27] We tested the existence of gender and ethnic differences in the influences of genetic and environmental factors on $U_{NE}V$ and U_EV, as described in detail elsewhere.[28]

Bivariate modeling of twin data. A bivariate Cholesky decomposition was used to model the covariance between $U_{NE}V$ and U_EV.[29] This model allows determination of the extent to which the covariance (or phenotypic correlation, r_p) can be explained by genetic or environmental factors influencing both traits. Genetic and environmental correlations between two traits can be calculated. The genetic correlation (r_g) between two traits gives an indication of the amount of overlap between (sets of) genes influencing those traits. r_g is calculated as the (additive) genetic covariance (COV_A) between two traits divided by the square root of the product of the total genetic variance components (V_A) of each of the traits. The genetic correlation between two traits therefore equals: $r_g = COV_A\,(\text{trait 1, trait 2}) / \sqrt{(V_A\text{trait 1} * V_A\text{trait 2})}$. Common and unique environmental correlations (r_c and r_e, respectively) are calculated in a similar fashion. The genetic and environmental factor loadings and correlations can be used to calculate the proportion of the phenotypic correlation explained by genetic and environmental factors.

The bivariate Cholesky decomposition is shown in Figure 2. Estimates for the path coefficients, that is, the model parameters (for example, a_{11}, c_{11}, e_{11}), are obtained by using a fit function that minimizes the difference between the observed covariance matrix and the expected covariance matrix implied by the model. In a model without dominance effects, the relative contribution of genetic variance to the total variance in U_EV, also known as its heritability, is the effect of the additive genetic factor A, and is obtained as the ratio $a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$. The heritability of $U_{NE}V$ is the summed effect of the genetic factors A1 and A2, and is obtained as the ratio $(a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+c_{21}^2+c_{22}^2)$. The percentage of total $U_{NE}V$ variance caused by genetic effects also influencing U_EV is calculated as $a_{21}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+c_{21}^2+c_{22}^2)$. Finally, the percentage of total $U_{NE}V$ variance caused by genetic effects that are specific to $U_{NE}V$ is equal to $a_{22}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+c_{21}^2+c_{22}^2)$.

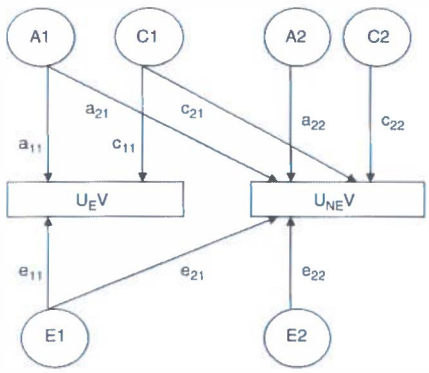

Figure 2 Path diagram for a bivariate model. For clarity only one twin is depicted. A1, A2 = Genetic variance components; C1, C2 = common environmental variance components; E1, E2 = unique environmental variance components; a_{11} through a_{22} = genetic path coefficients (or factor loadings) of which a_{22} represents specific genetic influences on $U_{NE}V$; c_{11} through c_{22} = common environmental path coefficients (or factor loadings) of which c_{22} represents specific common environmental influences on $U_{NE}V$; e_{11} through e_{22} = unique environmental path coefficients (or factor loadings) of which e_{22} represents specific unique environmental influences on $U_{NE}V$. Formula for the different heritability estimates are as follows:

h^2 total $(U_EV) = a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$

h^2 total $(U_{NE}V) = (a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

h^2 shared ($U_{NE}V$ explained by U_EV) $= a_{21}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

h^2 specific $(U_{NE}V) = a_{22}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

Before the analysis, $U_{NE}V$ and U_EV were log-transformed to obtain a better approximation of the normal distribution. Mean values of log-transformed $U_{NE}V$ and U_EV were adjusted for age, gender, ethnicity and body size (that is, BSA) before using the residuals in model fitting. Deterioration in model fit, after each term was dropped from the full model, was assessed to determine the significance of variance components A and C (or D). Standard hierarchic χ^2-tests were used to select the best fitting models in combination with Akaike's information criterion (AIC = χ^2-2 df). The model with the lowest AIC reflects the best balance of goodness of fit and parsimony[27]. Effects of gender, ethnicity and their interaction (ethnicity x gender) on mean values were tested while adjusting for age using generalized estimating equations. Generalized estimating equations take the non-independence between twins into account and yields unbiased standard errors and *P*-values.[30] Preliminary analyses and generalized estimating equationss were performed using STATA 10.0 (Stata Corp., College Station, TX, USA). Genetic modeling was carried out with Mx, a computer program specifically designed for the analysis of twin and family data.[31]

RESULTS

Sample and Demographics

Table 1 shows the general characteristics stratified for ethnicity and gender. Males were taller and heavier than females, but the weight difference was larger in European Americans. Compared with their male counterparts, BMI was larger in African-American females but smaller in European-American females. Neither $U_{NE}V$ nor U_EV showed significant effects of ethnicity, gender, age or BSA. None of the traits showed significant differences between MZ and DZ twins.

Cross-trait correlations of office and 24-h, daytime and night-time ambulatory BP with $U_{NE}V$ and U_EV are shown in Table 2. $U_{NE}V$ and U_EV were highly correlated in both European Americans ($r = 0.77$; 0.85 and 0.62 in males and females, respectively) and African Americans ($r = 0.84$; 0.86 and 0.80 in males and females, respectively). There was no clear pattern for correlations of BP measures with either $U_{NE}V$ or U_EV in European Americans. However, in African Americans overall moderately negative correlations were observed, which were especially prominent in African-American females for 24-h and daytime ambulatory BP values.

Table 3 presents the twin correlations for $U_{NE}V$ and U_EV of each zygosity group in European and African Americans as well as in the overall sample. MZ correlations showed consistently higher values than DZ correlations, indicating an important contribution of genetic factors. The DZ correlations of $U_{NE}V$ were less than half of the corresponding MZ correlations, suggesting presence of dominance (D) effects.

Table 1 General characteristics, U_EV and $U_{NE}V$ of 101 European Americans and 91 African Americans

	European Americans		*African Americans*		*Ethnicity and gender effects*		
	Males	*Females*	*Males*	*Females*	*Ethnicity P*	*Gender P*	*Ethnicity × Sex P*
N, individuals	52	49	55	36	—	—	—
Age, year	18.7 ± 3.9	18.6 ± 2.8	17.5 ± 2.6	17.0 ± 2.6	NS	NS	NS
Height, m	1.76 ± 0.08	1.62 ± 0.07	1.76 ± 0.09	1.64 ± 0.06	NS	<0.001	NS
Weight, kg	75.9 ± 17.9	62.9 ± 17.8	75.5 ± 19.8	74.9 ± 22.5	NS	0.001	0.016
BMI, kgm^{-2}	24.4 ± 4.8	23.7 ± 5.6	24.3 ± 5.3	27.7 ± 7.8	NS	NS	0.025
U_EV, μgh^{-1}	0.23 ± 0.23	0.16 ± 0.12	0.29 ± 0.31	0.25 ± 0.23	NS	NS	NS
$U_{NE}V$, μgh^{-1}	1.86 ± 1.33	1.58 ± 0.71	2.07 ± 1.54	1.96 ± 1.50	NS	NS	NS

Abbreviations: BMI, body mass index; NS, not significant; U_EV, urine epinephrine excretion rate; $U_{NE}V$, urine norepinephrine excretion rate.
Values are mean ± s.d. unless stated otherwise.
Weight, BMI, U_EV and $U_{NE}V$ were log-transformed before analysis.

Table 2 Correlations of $U_{NE}V$ and U_EV with office BP, and 24 h, daytime and night time ambulatory BP

	European American overall (male/female)		*African American overall (male/female)*	
N, individuals	*101(52/49)*		*91(55/36)*	
Measures	$U_{NE}V$	U_EV	$U_{NE}V$	U_EV
U_EV	**0.77(0.85/0.62)**		**0.84(0.86/0.80)**	
Office SBP	−0.18(−0.22/−0.11)	−0.13(−0.18/−0.10)	**−0.23(−0.31/−0.24)**	**−0.21(−0.24/−0.32)**
Office DBP	0.002(−0.08/0.12)	0.03(−0.14/0.18)	0.04(−0.05/−0.002)	0.08(−0.10/−0.02)
24 h SBP	−0.08(−0.11/−0.11)	−0.10(−0.10/−0.20)	−0.18(−0.10/−0.56)	−0.22(−0.03/−0.73)
24 h DBP	0.12(0.15/0.18)	0.06(0.13/−0.32)	0.24(0.08/−0.51)	**−0.33(−0.06/−0.61)**
Daytime SBP	−0.06(−0.11/−0.09)	−0.07(−0.08/−0.17)	−0.14(0.06/−0.57)	−0.20(0.11/−0.79)
Daytime DBP	0.18(0.18/0.25)	0.01(0.15/−0.17)	0.22(0.16/−0.53)	0.27(0.08/−0.63)
Night time SBP	−0.13(−0.20/−0.10)	−0.19(−0.24/−0.18)	−0.19(−0.35/−0.38)	−0.18(−0.29/−0.34)
Night time DBP	0.02(0.01/0.04)	0.17(0.06/−0.36)	0.17(−0.16/−0.28)	−0.27(−0.35/−0.21)

Abbreviations: DBP, diastolic BP; SBP, systolic BP; U_EV, urine epinephrine excretion rate; $U_{NE}V$, urine norepinephrine excretion rate.
$U_{NE}V$ and U_EV were log transformed and adjusted for BSA.
All BP measures were adjusted for age and BMI before the analysis.
There are 29 missing values in European American males, 26 in European American females, 34 in African American males and 17 in African American females for the ambulatory BP measures.
Significant correlations ($P<0.05$) are shown in bold.

Table 3 Twin correlations for each zygosity group in European and African Americans

	European Americans		*African Americans*		*Overall*	
Measures	*MZ*	*DZ*	*MZ*	*DZ*	*MZ*	*DZ*
Pairs, *N*	23	22	24	15	47	37
U_EV, $\mu g\,h^{-1}$	0.69	0.54	0.67	0.42	0.68	0.48
$U_{NE}V$, $\mu g\,h^{-1}$	0.75	0.39	0.59	0.20	0.67	0.28

Abbreviations: DZ, dizygotic twins; MZ, monozygotic twins.
$U_{NE}V$, urine norepinephrine excretion rate; U_EV, urine epinephrine excretion rate.
$U_{NE}V$ and U_EV were log transformed and adjusted for age, ethnicity, sex and BSA before analysis.

Univariate modeling

Parameter estimates of the best fitting models for $U_{NE}V$ and U_EV are shown in Table 4. Variance component estimates were collapsed across gender and ethnicity, because genetic and environmental parameter estimates were not significantly different between males and females or between European and African Americans (data not shown). Both traits were significantly heritable with heritabilities of 68% (50–79%) for $U_{NE}V$ and 74% (59–83%) for U_EV. The model including additive genetic and unique environmental effects without gender or ethnicity differences provided the best fit. That is, dropping common environmental (C) or dominant genetic (D) effects had virtually no effect on model fit indicating they do not contribute significantly.

Bivariate model

Subsequently, we performed bivariate model fitting to estimate to which extent phenotypic correlations can be explained by genetic or environmental factors that influence both $U_{NE}V$ and U_EV (Figure 2). As the bivariate model can also be used to estimate the variance components for each individual trait, we found very similar estimates of heritability as in the univariate model (Figure 3). The phenotypic correlation between $U_{NE}V$ and U_EV was high ($r_p = 0.81$). The genetic correlation between $U_{NE}V$ and U_EV was even higher at 0.86, while unique environmental correlations were somewhat lower but still highly significant ($r_e = 0.71$) (Figure 3). Not surprisingly, decomposition of the phenotypic correlation into its genetic and environmental parts showed it to be largely (73%) due to genetic factors.

Figure 4 presents sources of variance of $U_{NE}V$ based on the best-fitting bivariate model. Eighteen percent of the total variance of $U_{NE}V$ could be attributed to specific genetic factors that only influence $U_{NE}V$. Genetic factors that also influence U_EV contributed to the total variance for $U_{NE}V$ to a large extent (50%). Comparatively, environmental factors that also influenced U_EV contributed substantially less to the total variance of $U_{NE}V$ (16%).

DISCUSSION

The present study shows significant heritabilities for $U_{NE}V$ (0.68, 95% CI: 0.50–0.79) and U_EV (0.74, 95% CI: 0.59–0.83) using the best-fitting univariate model. The genetic correlation was 0.86 (95% CI: 0.76–0.97), indicating a large overlap in the genes influencing $U_{NE}V$ and U_EV. Fifty percent of the variance in $U_{NE}V$ was explained by genes that also influence individual differences in U_EV.

Several groups have investigated the heritability of catecholamines in blood and urine. For instance, Williams *et al.*[5] reported substantial heritability (h^2) estimates of blood NE ($h^2 = 57\%$) and E levels ($h^2 = 74\%$ for males and $h^2 = 64\%$ for females), based on data of 109 twin pairs. In addition, Jedrusik *et al.*[12] studied 39 MZ twin pairs and 37 age-matched same-gender DZ twin pairs to determine the effects of genetic factors on sympathetic activity in twins. Catecholamines in blood and urine were used as measures of sympathetic activity, and genetic contributions were 42 and 76% for NE and 69 and 65% for E, respectively. Finally, Zhang *et al.*[14] found that heritabilities for plasma and urinary E or NE ranged from 0.33 to 0.61 in a sample of Caucasian twins.[14] Similar results were reported more recently in a slightly larger sample showing that both plasma and urinary E and NE were significantly heritable and ranged from 0.49 to 0.72.[6,13]

We are not aware of other heritability studies estimating the basal sympathetic tone by means of overnight urinary excretion rates of both NE and E. In principle, catecholamine levels in blood may more accurately reflect sympathetic activity than overnight urinary excretion rates of NE and E. However, collection of overnight urine for measurement of catecholamine excretion rates has a number of advantages. Catecholamine excretion rates provide an integrated measure of 'steady-state' operating levels of the sympathetic nervous

Table 4 Parameter estimates and 95% CIs of best-fitting univariate models

	h^2 (95%CI)	c^2 or d^2 (95%CI)	e^2 (95%CI)	2LL	df	$\Delta\chi^2$	Δdf	P	AIC
U_EV, μg/h									
ACE	0.60 (0.13–0.83)	0.13 (0.00–0.54)	0.27 (0.17–0.43)	440.685	183				
AE	**0.74 (0.59–0.83)**		**0.26 (0.17–0.41)**	**440.945**	**184**	**0.259**	**1**	**0.611**	**1.741**
CE		0.59 (0.43–0.71)	0.41 (0.29–0.57)	446.891	184	6.206	1	0.013	4.206
E	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.00 (1.00–1.00)	480.582	185	39.896	2	0.000	35.896
$U_{NE}V$, μg/h									
ADE	0.45 (0.00–0.79)	0.23 (0.00–0.79)	0.32 (0.21–0.50)	355.981	183				
AE	**0.68 (0.50–0.79)**		**0.32 (0.21–0.50)**	**356.136**	**184**	**0.155**	**1**	**0.694**	**1.845**
E	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.00 (1.00–1.00)	386.584	185	30.603	2	0.000	26.603

Abbreviations: $U_{NE}V$, urine norepinephrine excretion rate; U_EV, urine epinephrine excretion rate; h^2, heritability; c^2, common environmental variance; d^2, dominant genetic variance; e^2, unique environmental variance; CI, confidence interval.
U_EV and $U_{NE}V$ were log-transformed and adjusted for age, ethnicity, sex and BSA prior to analysis.
Best fitting models are in bold.

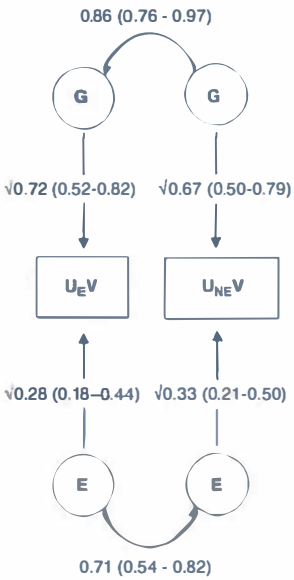

Figure 3 Best-fitting bivariate model for U_EV and $U_{NE}V$. For clarity, only one twin is depicted. Factor loadings (or path coefficients) are expressed as square roots (± their 95% confidence intervals) to make clear that squaring those factor loadings yields estimates of genetic and environmental variance components as shown in text. The genetic (r_g) and environmental (r_e) correlations between U_EV and $U_{NE}V$ are shown above and below the double-headed arrows. *A* indicates additive genetic factor; *E*, unique environmental factor.

system, capturing the more chronic effects of stress and have been widely used in studies of stress and CVD risk,[32–38] including McEwen and Seeman's allostatic load studies.[32,33] Furthermore, overnight urine can be reliably collected by adolescents, and the potential confounding effects of physical activity are minimized because subjects generally spend this time at home, mostly in bed.[39] Thus, we expected that overnight urinary excretion rates of NE and E might be less influenced by environmental factors such as mental stress and physical exercise, making it easier to delineate the genetic contribution to basal sympathetic activity. This is indeed supported by our present results, showing heritabilities on par or exceeding those previously reported for blood[5,42] and urinary [12] catecholamines measured during the daytime. Urinary catecholamine levels in the studies by Zhang *et al.*[14] and Rao *et al.*[6,40,41] were normalized for creatinine excretion in the same sample, but it is not clear whether 24 h, overnight or spot urine was used, which complicates meaningful comparisons with our study.

Several studies have reported associations between genetic polymorphisms and catecholamine secretion. One study found that the Gly364Ser polymorphism in the catecholamine storage vesicle protein chromogranin A gene *CHGA* displayed diminished inhibition of catecholamine secretion from cultured neurons. Renal NE excretion was diminished by around 26% and E excretion by around 34% in Gly/Ser heterozygotes.[40] Another study performed in a similar sample suggested that common tyrosine hydroxylase (*TH*) promoter polymorphisms with variants at C-824T and A-58IG showed significant associations with urinary catecholamine excretion.[41] C-824T also exerted significant pleiotropic effects on the coupling between blood pressure response to cold stress and urinary NE. In addition, the second most frequent promoter haplotype (TGGG), based on four common promoter SNPs (C-824T, G-801C, A-581G, and G-494A) displayed copy number-dependent effects on urinary E ($P = 0.0044$, % variance explained = 5.7%) and NE excretion ($P = 0.0125$, % variance explained = 4.06%). This haplotype also showed pleiotropy, increasing both NE excretion and blood pressure during stress.[6]

Using the same Georgia Cardiovascular Twin cohort, we have performed several candidate gene studies investigating genes in the sympathetic nervous system pathway, including adrenergic receptor and signal transduction genes, for association with BP regulation and hypertension risk.[18,20] Polymorphisms of genes coding for the enzymes involved in synthesis and degradation of catecholamines could also be involved. For instance, *TH* coding for tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, was found to be associated with essential hypertension in a case-control study.[42] Another example is phenylethanolamine *N*-methyltransferase (*PNMT*), coding for the terminal enzyme of the catecholamine synthetic pathway, which catalyzes the synthesis of E from NE. Methods in comparative genomics have identified a genetic locus associated with BP regulation in the stroke-prone spontaneously hypertensive rat on rat chromosome 10 in a conserved syntenic group that corresponded to a gene encoding *PNMT* on chromosome 17q21-q22 in humans.[43]

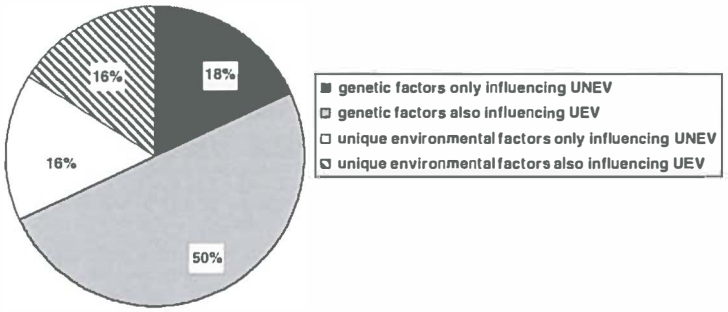

Figure 4 A decomposition of the variance of $U_{NE}V$ in its genetic and environmental components (that is, genetic and environmental sources of individual differences in $U_{NE}V$) is shown. Because we used a bivariate model in which both $U_{NE}V$ and U_EV were included, we could further discriminate between genetic and environmental factors that also influenced U_EV or were specific to $U_{NE}V$. Results are those of the best-fitting bivariate model as shown in Figure 2.

It is likely that many genes in the sympathetic system are involved in heritable NE and E excretion and these may also contribute to essential and stress-induced hypertension. Previously, we have proposed a model wherein chronic environmental stress in concert with genetic predisposition and factors, such as gender and ethnicity, might eventually lead to essential hypertension, type 2 diabetes and cardiovascular disease.[18,44] The sympathetic system is a key component of this model and the current study suggests that genetic factors are strong determinants of basal sympathetic activity. On the basis of this model, we expected positive correlations of $U_{NE}V$ and U_EV with BP measures, which is not what we observed. In spite of our wide array of BP measures, no associations were found in European Americans and, if anything, correlations in African Americans were moderately negative. One possible explanation of this latter result may be that those African Americans excreting more catecholamines are also better capable of (down) regulating their BP, for example, through more efficient sodium excretion and volume regulation.

Several limitations need to be recognized. First, as the Georgia Cardiovascular Twin Study is comprised of youth and young adults, the generalizability of these results to other adult populations remains to be determined. Second, we did not have information on the quality of the previous night's sleep. The prospect of participating in our study the next day may have caused some anticipatory stress, which could have affected sleep quality and $U_{NE}V$ and U_EV. Finally, not all subjects with excretion rates also had ambulatory BP measures available (Table 2). Further studies with larger sample sizes are warranted to more definitively determine the relation between overnight urinary excretion rates of NE and E and BP.

In summary, individual differences in both $U_{NE}V$ and U_EV and the association between them are substantially heritable, indicating that measurements of $U_{NE}V$ and U_EV provide a viable method for the study of sympathetic tone in genetic epidemiological research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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CHAPTER 5

Genetic influences on cardiovascular stress reactivity

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Review

Genetic influences on cardiovascular stress reactivity

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ABSTRACT

Individual differences in the cardiovascular response to stress play a central role in the reactivity hypothesis linking frequent exposure to psychosocial stress to adverse outcomes in cardiovascular health. To assess the importance of genetic factors, a meta-analysis was performed on all published twin studies that assessed heart rate (HR) or blood pressure (BP) reactivity to the cold pressor test or various mental stress tasks. For reactivity to mental stress, the pooled heritability estimate ranged from 0.26 to 0.43. Reactivity to the cold pressor test yielded heritability estimates from 0.21 to 0.55. An ensuing review of genetic association studies revealed a number of genes, mostly within the sympathoadrenal pathway, that may account for part of the heritability of cardiovascular stress reactivity. Future progress in gene finding, that should include measures of sympathetic and vagal stress reactivity, may help uncover the molecular pathways from genetic variation to stress reactivity.

Contents

1. Introduction

Twin research has suggested a clear-cut genetic contribution to cardiovascular disease (CVD). These studies typically compare the concordance rates for cardiovascular morbidity or mortality in monozygotic (MZ) twins to those in dizygotic (DZ) twins. MZ twins, with a few rare exceptions (Martin et al., 1997), share all of their genotypes, whereas DZ twins on average share only half of the genotypes segregating in the family. Therefore, a larger concordance for CVD in MZ than in DZ twins means that genetic variation contributes to the risk for CVD. A landmark paper was published by twin researchers in Sweden (Marenberg et al., 1994). They searched the National Death Registry for death certificates on ~21,000 twins born in Sweden between 1886 and 1925, where both twins within a pair still lived within the country in 1961. Survival analysis in males showed that the relative hazard of death from coronary heart disease when one's twin died of coronary heart disease before the age of 55 years, as compared with the hazard when one's twin did not die before 55, was 8.1 for monozygotic twins and 3.8 for male dizygotic twins. Among the women, when one's twin died of coronary heart disease before the age of 65 years, the relative hazard was 15.0 for monozygotic twins and 2.6 for dizygotic twins. Re-analysis using a correlated frailty model, which translates discrete yes/no traits into a continuously distributed latent liability, yielded a heritability to die from coronary heart disease of 57% in males and 38% in females (Zdravkovic et al., 2002, 2004).

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The genetic contribution to cardiovascular disease endpoints most likely results from the joint effects of risk genes on the classical biological and behavioral risk factors that impact on the atherosclerotic process. These include smoking (Li et al., 2003; Vink et al., 2005), physical inactivity (Stubbe et al., 2006; Beunen and Thomis, 1999), body mass index (Schousboe et al., 2003; Silventoinen et al., 2003) diabetes (Poulsen et al., 1999), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Evans et al., 2003; Kupper et al., 2005b), and plasma LDL-C and HDL-C levels (Beekman et al., 2002). Heritability estimates for these established risk factors are 50% or higher in most adult twin samples and these estimates remain remarkably similar across the adult life span (Hottenga et al., 2005, 2006; Snieder et al., 1999). Population variance in a number of other suspected risk factors, including insulin resistance (Poulsen et al., 2001; Liu et al., 2009; Simonis-Bik et al., 2008), inflammation (Worns et al., 2006; Su et al., 2008), hemostasis (de Lange et al., 2006; Peetz et al., 2004), cardiac autonomic control (Wang et al., 2009; Kupper et al., 2005a, 2006), type A (Rebollo and Boomsma, 2006) or type D (Kupper et al., 2007) personality, and depression (Sullivan et al., 2000) has also shown substantial genetic variation.

In addition to the above risk factors, cardiovascular reactivity to mental and emotional stressors has long been regarded to be a potential contributor to individual differences in cardiovascular disease risk (Treiber et al., 2003b; Kamarck and Lovallo, 2003). A propensity towards exaggerated reactivity combined to frequent exposure to stress may lead to allostatic changes in many of the regulatory systems important in CVD and identified above, e.g. blood pressure regulation, lipid and insulin metabolism, inflammation, and hemostasis. Cardiovascular stress reactivity is typically assessed by comparing baseline levels of heart rate (HR), SBP, and DBP to the levels attained during deliberate exposure to a painful stimulus like the cold pressor test or to mentally demanding tasks that are made stressful by adding performance-contingent reward or punishment (electric shock, loud noise). Apart from HR, SBP, and DBP additional measures are sometimes assessed as well to establish the relative contribution of the sympathetic versus the parasympathetic nervous system or vascular versus cardiac responses to the observed changes in HR and BP. These measures include venous or arterial catecholamine levels, pre-ejection period (PEP), heart rate variability (HRV), stroke volume, and total peripheral resistance (Berntson et al., 2008; Lawler et al., 2001; Sherwood et al., 1990).

Psychometric studies have established satisfactory temporal stability of the commonly used cardiovascular reactivity measures, particularly when aggregated over multiple stressors (Kamarck et al., 1992; Swain and Suls, 1996). Individual differences in cardiovascular reactivity to laboratory stress have been shown to translate well to naturalistic settings (Kamarck et al., 2003) and prospective studies have shown that these individual differences predict future hypertension (Light et al., 1999; Flaa et al., 2008; Moseley and Linden, 2006; Matthews et al., 2004; Newman et al., 1999) and atherosclerosis (Kamarck et al., 1997; Matthews et al., 2006). Obvious next questions are to what extent these individual differences in cardiovascular reactivity to stress are heritable and which genes may be involved. Identifying the genetic factors influencing stress reactivity may greatly improve the precision of epidemiological studies linking psychosocial stress to disease outcome (Yusuf et al., 2004). By lumping together subjects who are genetically susceptible to the effects of psychosocial stressors with those subjects that are less susceptible, many previous studies in the field may even have underestimated the significance of negative health effects in the former susceptible group.

Already more than a decade ago, Turner and Hewitt (1992; Hewitt and Turner, 1995) reviewed a number of early twin studies that explored the genetic and environmental origins of individual differences in HR and BP reactivity to psychological challenge. Their conclusion was that HR and BP reactivity are substantially heritable. Additional twin studies of cardiovascular reactivity have since confirmed heritability of HR and BP reactivity, but estimates for DBP, SBP and HR reactivity have been very different across studies for the same task or, within the same study, across different tasks, and have ranged from 0.00 to 0.85 (Ditto, 1993; Lensvelt-Mulders and Hettema, 2001; Smith et al., 1987; Carmelli et al., 1991; McIlhany et al., 1975; de Geus et al., 2007; Li et al., 2001; McCaffery et al., 2002).

Here we performed a meta-analysis on all published studies in twins that assessed HR or BP reactivity to the cold pressor or mental stress tasks. In the discussion, we briefly review the heritability estimates of a number of other cardiovascular measures for which sufficient numbers were not yet available to do a meta-analysis. We further review the first attempts to find genetic associations with reactivity measures in molecular genetic studies.

2. Methods

2.1. Search strategy

We identified articles on cardiovascular stress reactivity in twins through a systematic search of the MEDLINE (Pubmed) database and inspection of reference lists of selected articles up to July 1st 2009. The following terms were used for the MEDLINE search ("Blood Pressure"[Mesh] OR "heart rate"[Mesh]) AND "Twin"[All Fields] AND "Heritability"[All Fields] AND ("reactivity"[All Fields] OR "response"[All Fields] OR "stress"[All Fields]). No language restriction was applied for searching and study inclusion.

We only included the articles that reported the sample size and separate correlations for monozygotic (MZ) and dizygotic (DZ) twins for SBP, DBP or HR reactivity to the cold pressor test or to mentally demanding tasks. Three articles by Busjahn et al. (1996), Snieder et al. (1997) and Carmelli et al. (1985) were preliminary reports of the same studies as reported in Li et al. (2001), de Geus et al. (2007) and Carmelli et al. (1991), respectively. The initial reports were removed as duplicates because the latter articles reported larger sample sizes. We further excluded literature reviews and three older studies in which the sample size was less than 80 twin pairs (Shapiro et al., 1968; Carroll et al., 1985; Turner et al., 1986). If the results from multiple independent samples ($\geq$80 pairs) were included in a single paper, these were treated as separate studies (e.g. adolescent and middle-aged twin samples in de Geus et al., 2007). For the cold pressor test, we also included the study by Snieder et al. (1997; van Doornen et al., 1998) for which the twin correlations have not been published previously. For each included study, we listed authors, publication year, and extracted information on ethnicity, sample size, mean age with standard deviation and age range, stressors and twin correlations for SBP, DBP, and HR (see Tables 1 and 4).

2.2. Meta-analysis

Meta-analysis was done separately for reactivity to mental stress and the cold pressor test. In studies that used multiple mental stressors, we used the aggregated reactivity measures across all mental stressors. Two studies already reported on aggregated mental stress only (McCaffery et al., 2002; de Geus et al., 2007). In a third study (Ditto, 1993) we aggregated the two mental stress tasks by estimating a single twin correlation across these tasks during the pooling procedure described below.

Structural equation modeling (SEM) in Mx software (Neale et al., 2006) was used to estimate five pooled twin correlations across all studies for five zygosity-by-sex groups: MZ males (MZM), DZ males (DZM), MZ females (MZF), DZ females (DZF) and dizygotic opposite sex (DOS). Two studies originally reported sample size and twin correlations in only two zygosity groups (MZ and DZ)

Table 1
Description of twin correlations of SBP, DBP and HR reactivity to mental stress.

Investigator	Ethnicity	Sample size (pairs)	Age		Stressors	Twin correlations		
			Mean ± SD	Range		SBP	DBP	HR
Smith et al. (1987)	Caucasians	82 MZM 88 DZM	35.0 ± n.a.	21.0–61.0	MA	R_{MZM}: 0.24 R_{DZM}: −0.06[a]	R_{MZM}: 0.30 R_{DZM}: 0.04	R_{MZM}: 0.07 R_{DZM}: 0.21
Carmelli et al. (1991)	Caucasians	47 MZM 54 DZM	62.4 ± n.a.	59.0–69.0	MA	R_{MZM}: 0.71 R_{DZM}: 0.31	R_{MZM}: 0.56 R_{DZM}: 0.23	R_{MZM}: 0.46 R_{DZM}: 0.17
Ditto (1993)	Caucasians	20 MZM 20 MZF 20 DZM 20 DZF 20 DOS	20.0 ± 5.0	12.0–44.0	Aggregated 1. MA 2. CT	R_{MZM}: 0.32 R_{DZM}: −0.08 R_{MZF}: 0.23 R_{DZF}: 0.16 R_{DOS}: 0.19	R_{MZM}: 0.65 R_{DZM}: 0.03 R_{MZF}: 0.19 R_{DZF}: 0.20 R_{DOS}: 0.19	R_{MZM}: 0.59 R_{DZM}: 0.07 R_{MZF}: 0.50 R_{DZF}: 0.12 R_{DOS}: 0.15
Lensvelt-Mulders and Hettema (2001)	Caucasians	57 MZF 43 DZF	31.5 ± n.a.	18.0–47.0	Film evoked emotion	R_{MZF}: 0.44 R_{DZF}: 0.27	R_{MZF}: 0.27 R_{DZF}: 0.23	n.a.
Li et al. (2001)	Caucasians	82 MZM 42 MZF 37 DZM 13 DZF 24 DOS	30.0 ± 12.0	n.a.	MA	R_{MZM}: 0.05 R_{DZM}: 0.21 R_{MZF}: 0.53 R_{DZF}: 0.18 R_{DOS}: 0.39	R_{MZM}: 0.20 R_{DZM}: 0.20 R_{MZF}: 0.38 R_{DZF}: 0.003 R_{DOS}: 0.33	R_{MZM}: 0.53 R_{DZM}: 0.28 R_{MZF}: 0.61 R_{DZF}: 0.42 R_{DOS}: 0.36
McCaffery et al. (2002)	Caucasians	54 MZM 47 MZF 22 DZM 22 DZF	21.1 ± 2.8	18.0–30.0	Aggregated 1. Stroop 2. MA	R_{MZM}: 0.43 R_{DZM}: −0.19 R_{MZF}: 0.24 R_{DZF}: 0.20	R_{MZM}: 0.37 R_{DZM}: −0.19 R_{MZF}: 0.24 R_{DZF}: −0.09	R_{MZM}: 0.56 R_{DZM}: −0.05 R_{MZF}: 0.49 R_{DZF}: 0.46
de Geus et al. (2007)	Adolescent Caucasians	35 MZM 35 MZF 31 DZM 30 DZF 29 DOS	16.7 ± 2.0	13.0–22.0	Aggregated 1. RT 2. MA	R_{MZM}: 0.56 R_{DZM}: 0.24 R_{MZF}: 0.24 R_{DZF}: 0.41 R_{DOS}: 0.04	R_{MZM}: 0.12 R_{DZM}: 0.11 R_{MZF}: 0.15 R_{DZF}: 0.27 R_{DOS}: −0.06	R_{MZM}: 0.37 R_{DZM}: 0.01 R_{MZF}: 0.50 R_{DZF}: 0.26 R_{DOS}: 0.27
	Middle-aged Caucasians	45 MZM 49 MZF 37 DZM 39 DZF 39 DOS	44.2 ± 6.7	34.0–63.0	Aggregated 1. RT 2. MA	R_{MZM}: 0.38 R_{DZM}: −0.19 R_{MZF}: 0.25 R_{DZF}: −0.16 R_{DOS}: 0.39	R_{MZM}: 0.14 R_{DZM}: 0.11 R_{MZF}: 0.27 R_{DZF}: −0.06 R_{DOS}: 0.07	R_{MZM}: 0.45 R_{DZM}: 0.45 R_{MZF}: 0.44 R_{DZF}: 0.11 R_{DOS}: 0.15

RT: reaction time task; MA: mental arithmetic task; CT: concept task; Stroop: color-word conflict task; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females; DOS: dizygotic opposite sex; n.a.: data not given in the article.
[a] The minus sign was incorrectly omitted in the original article as indicated by Turner and Hewitt (1992).

rather than five zygosity-by-sex groups (Li et al., 2001; McCaffery et al., 2002). We contacted the authors of these two studies, and both groups were willing and able to revisit their original dataset and provide us with the correlations for each zygosity-by-sex group.

For each zygosity-by-sex group, heterogeneity of the twin correlations across studies was tested by comparing the model that fixed the correlations to be equal across studies to the full model that estimated the twin correlations separately for each study. The degrees of freedom for this test is the number of the studies available for pooling minus one. Taking SBP reactivity to mental stress as an example (Table 1), MZM and DZM correlations were set equal across seven studies (there were only females in the study of Lensvelt-Mulders and Hettema (2001), MZF and DZF correlations across six studies (there were only males in studies of Smith et al. (1987) and Carmelli et al. (1991), and DOS correlations across three studies (Ditto, 1993 and adolescent and middle-aged twins in de Geus et al., 2007). To test for heterogeneity the fit of these models was then compared to the full models, with degrees of freedom of 6, 5, and 2 respectively.

2.2.1. Genetic modeling and sex differences

In a next step, SEM of the pooled twin correlations was used to estimate the genetic and environmental sources of individual differences (i.e. variance components) in BP and HR reactivity to mental stress and the cold pressor test. The sample size for each zygosity-by-sex group was equal to the sum of the sample sizes of all included studies. The full model allows for additive genetic (A), either common environmental (C) or dominant genetic (D) influences as well as unique environmental (E) influences on SBP, DBP and HR reactivity. The total variance was constrained to be equal to one in these models. More parsimonious models then leave out individual genetic or environmental components and we tested the loss of fit to the observed data by calculating the change in χ^2 ($\Delta\chi^2$) against the gain of degrees of freedom (Δdf).

The existence of sex differences in the influences of genetic and environmental factors on the phenotype can take several forms (Reynolds and Hewitt, 1995). Sex differences were examined by comparing a full model in which parameter estimates were allowed to differ in magnitude between males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. In addition, models were tested in which genetic or common environmental influences differ in kind between males and females. In this case, correlations in DOS twin pairs between the latent genetic (r_g) or common environmental (r_c) factors will be smaller than the normal values of 0.5 and 1, respectively.

3. Results

We identified a total of 20 potentially relevant articles through our searches, but excluded 12 for the reasons listed in Fig. 1. In the remaining eight articles, we identified nine published studies that met our inclusion criteria and added one previously unpublished analysis of cold pressor data in one of our own samples. Eight studies could be used in the meta-analysis of cardiovascular (CV) reactivity to mental stress (Table 1), and five could be used in the meta-analysis of cardiovascular reactivity to the cold pressor test (Table 4).

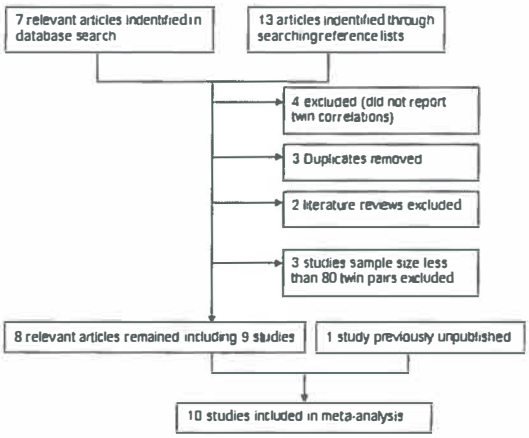

Fig. 1. Selection tree for the studies included in the meta-analysis. The database search identified articles up to July 1, 2009.

3.1. Cardiovascular reactivity to mental stress

Table 2 shows the total sample size and the pooled twin correlations for each zygosity-by-sex group. MZ correlations are consistently higher than DZ correlations for SBP, DBP as well as HR reactivity to mental stress, indicating an important contribution of genetic factors. Models that set twin correlations equal across studies in the five zygosity-by-sex groups did not have a significant worse fit (p's > 0.01) than the full model, with the exception of SBP reactivity in the MZ males ($p = 0.001$). This indicates heterogeneity in the MZM twin correlations across these studies. The main source of this heterogeneity was the study of Carmelli et al. (1991). Recomputing the pooled MZM correlation without this study changed the correlation estimate from 0.36 (0.26–0.45) to 0.29 (0.18–0.39).

Table 3 presents the genetic and environmental parameter estimates and 95% confidence intervals of the best fitting models for SBP, DBP and HR reactivity to mental stress. For SBP reactivity an AE model with sex differences in heritability provided the best fit. Heritabilities were 0.26 and 0.38 for SBP reactivity in males and females, respectively. Excluding the data of Carmelli et al. (1991) from the pooled MZM correlation decreased SBP reactivity in males to 0.19 (0.17–0.21).

For DBP reactivity, in addition to additive genetic effect (0.14), we also observed dominant genetic effects (0.15). There was no significant sex difference in the genetic and environmental estimates for DBP and HR.

3.2. Cardiovascular reactivity to the cold pressor test

Table 5 shows the total sample size and the pooled twin correlations for each zygosity-by-sex group. MZ correlations are higher than DZ correlations for SBP, DBP as well as HR reactivity, indicating an important contribution of genetic factors. Models that set twin correlations equal across studies in the five zygosity-by-sex groups did not fit significantly worse than the full model (p's > 0.01), with the exception of DBP reactivity in MZ males ($p = 2.0 \times 10^{-6}$) and HR reactivity in MZ females ($p = 3.6 \times 10^{-8}$). For DBP reactivity in MZ males, heterogeneity was mainly due to two studies (McIlhany et al., 1975; Ditto, 1993) that reported very high MZM correlations. Recomputing the pooled MZM correlations without these studies changed the DBP correlation estimate from 0.60 (0.50–0.68) to 0.48 (0.36–0.59). HR reactivity in MZ females was due to the study by Li et al. (2001). Excluding this study increased the pooled MZM correlation from 0.47 (0.30–0.62) to 0.68 (0.53–0.79).

Table 6 presents the genetic and environmental parameter estimates and the 95% confidence intervals of best fitting models for SBP, DBP and HR reactivity to the cold pressor test. For SBP reactivity, males and females showed significantly different heritabilities (0.21 versus 0.33). No sex differences were found for DBP and HR reactivity that showed heritabilities of 0.55 and 0.45, respectively. For HR reactivity, the r_g estimate was close to zero, indicating clear qualitative differences in the genetic influence on cold pressure reactivity in males and females. Excluding the most outlying data from the pooled twin correlations in the MZM group for DBP reactivity and in the MZF group for HR reactivity did not greatly affect the heritability estimates (data not shown).

Table 2
Pooled twin correlation estimates (95% CI) for five zygosity-by-sex groups for SBP, DBP and HR reactivity to mental stress.

	Sample size (pairs) MZM/DZM/MZF/DZF/DOS	MZM	DZM	MZF	DZF	DOS
SBP	365/289/250/167/112	0.36 (0.26, 0.45)[a]	0.05 (−0.07, 0.17)	0.34 (0.22, 0.45)	0.17 (0.02, 0.32)	0.27 (0.09, 0.44)
DBP	365/289/250/167/112	0.31 (0.21, 0.40)	0.10 (−0.02, 0.21)	0.26 (0.14, 0.37)	0.11 (−0.05, 0.26)	0.12 (−0.08, 0.30)
HR	365/289/193/124/112	0.42 (0.32, 0.50)	0.20 (0.08, 0.31)	0.51 (0.39, 0.61)	0.25 (0.07, 0.41)	0.23 (0.04, 0.40)

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females; DOS: dizygotic opposite sex.
[a] MZM correlation showed significant heterogeneity across studies ($p < 0.01$).

Table 3
Genetic and environmental parameter estimates (95% CI) of best fitting models for SBP, DBP and HR reactivity to mental stress.

	Best fitting models	A (95% CI)	D (95% CI)	E (95% CI)
SBP	AE sex difference: male	0.26 (0.23–0.29)	–	0.74 (0.71–0.77)
	AE sex difference: female	0.38 (0.33–0.43)	–	0.62 (0.57–0.67)
DBP	ADE no sex difference	0.14 (0.08–0.20)	0.15 (0.07–0.24)	0.71 (0.68–0.74)
HR	AE no sex difference	0.43 (0.40–0.47)	–	0.57 (0.53–0.60)

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; CI: confidence interval; A: additive genetic influence; D: dominant genetic influence; E: unique environmental influence

Table 4
Description of twin correlations of SBP, DBP and HR reactivity to the cold pressor test.

Investigator	Ethnicity	Sample size (pairs)	Age		Twin correlations		
			Mean±SD	Range	SBP	DBP	HR
McIlhany et al. (1975)	Caucasians and African Americans	40 MZM 47 MZF 32 DZM 36 DZF 45 DOS	14.0 ± 6.5	5.0–50.0	R_{MZM}: 0.58 R_{DZM}: 0.39 R_{MZF}: 0.55 R_{DZF}: 0.05 R_{DOS}: 0.09	R_{MZM}: 0.82 R_{DZM}: 0.46 R_{MZF}: 0.62 R_{DZF}: 0.39 R_{DOS}: 0.38	n.a.
Carmelli et al. (1991)	Caucasians	47 MZM 54 DZM	62.4 ± n.a.	59.0–69.0	R_{MZM}: 0.51 R_{DZM}: 0.42	R_{MZM}: 0.26 R_{DZM}: 0.34	R_{MZM}: 0.31 R_{DZM}: −0.04
Ditto et al. (1993)	Caucasians	20 MZM 20 MZF 20 DZM 20 DZF 20 DOS	20.0 ± 5.0	12.0–44.0	R_{MZM}: 0.65 R_{DZM}: 0.18 R_{MZF}: 0.38 R_{DZF}: 0.07 R_{DOS}: 0.04	R_{MZM}: 0.84 R_{DZM}: 0.19 R_{MZF}: 0.37 R_{DZF}: −0.08 R_{DOS}: 0.10	R_{MZM}: 0.50 R_{DZM}: 0.31 R_{MZF}: 0.78 R_{DZF}: 0.08 R_{DOS}: −0.04
Li et al. (2001)	Caucasians	82 MZM 41 MZF 37 DZM 13 DZF 22 DOS	30.0 ± 12.0	n.a.	R_{MZM}: 0.37 R_{DZM}: 0.37 R_{MZF}: 0.32 R_{DZF}: −0.28 R_{DOS}: 0.35	R_{MZM}: 0.49 R_{DZM}: 0.48 R_{MZF}: 0.45 R_{DZF}: 0.13 R_{DOS}: 0.03	R_{MZM}: 0.58 R_{DZM}: 0.38 R_{MZF}: −0.07 R_{DZF}: 0.32 R_{DOS}: 0.00
Snieder et al. (data collected in 1994) Previously unpublished	Caucasians	46 MZM 50 MZF 37 DZM 40 DZF 40 DOS	48.3 ± 6.6	30.0–58.0	R_{MZM}: 0.43 R_{DZM}: 0.36 R_{MZF}: 0.35 R_{DZF}: 0.29 R_{DOS}: 0.14	R_{MZM}: 0.65 R_{DZM}: 0.16 R_{MZF}: 0.54 R_{DZF}: 0.19 R_{DOS}: 0.15	R_{MZM}: 0.43 R_{DZM}: 0.42 R_{MZF}: 0.64 R_{DZF}: 0.09 R_{DOS}: −0.02

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females; DOS: dizygotic opposite sex.
n.a.: data not given in the article.

Table 5
Pooled twin correlation estimates (95% CI) for five zygosity-by-sex groups for SBP, DBP and HR reactivity to the cold pressor test.

	Sample size (pairs) MZM/DZM/MZF/DZF/DOS	MZM	DZM	MZF	DZF	DOS
SBP	235/180/158/109/127	0.47 (0.37, 0.57)	0.37 (0.23, 0.49)	0.41 (0.27, 0.53)	0.11 (−0.09, 0.29)	0.14 (−0.03, 0.31)
DBP	235/180/158/109/127	0.60 (0.50, 0.68)[a]	0.34 (0.20, 0.47)	0.52 (0.40, 0.63)	0.21 (0.01, 0.38)	0.21 (0.03, 0.37)
HR	195/148/111/73/82	0.48 (0.36, 0.58)	0.24 (0.07, 0.39)	0.47 (0.30, 0.62)[a]	0.13 (−0.11, 0.35)	0.02 (−0.24, 0.20)

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females; DOS: dizygotic opposite sex.
[a] Correlation showed significant heterogeneity across studies ($p < 0.01$).

Table 6
Genetic and environmental parameter estimates (95% CI) of best fitting models for SBP, DBP and HR reactivity to the cold pressor test.

	Best fitting models	A (95% CI)	C (95% CI)	E (95% CI)	r_g[a]
SBP	ACE sex difference: male	0.21 (0.04–0.35)	0.26 (0.15–0.41)	0.53 (0.45–0.60)	
	ACE sex difference: female	0.33 (0.28–0.40)	–	0.67 (0.60–0.72)	
DBP	AE no sex difference	0.55 (0.50–0.61)	–	0.45 (0.39–0.50)	
HR	AE no sex difference, r_g estimated	0.45 (0.40–0.51)	–	0.55 (0.49–0.60)	0.01

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; CI: confidence interval; A: additive genetic influence; C: common environmental influence; E: unique environmental influence.
[a] r_g is the correlation between additive genetic factors for DOS twins.

4. Discussion

Individual differences in the cardiovascular response to stress play a central role in the reactivity hypothesis linking frequent exposure to psychosocial stress to adverse outcomes in cardiovascular health (Treiber et al., 2003b; Kamarck et al., 2003). Here we used meta-analysis of twin resemblance in SBP, DBP and HR reactivity to show that cardiovascular stress reactivity to mental stressors and the cold pressor test are heritable traits. For SBP reactivity to the mental stressors, the pooled heritability across all studies ranged from 0.26 (males) to 0.38 (females). SBP reactivity to the cold pressor test yielded comparable heritability estimates ranging from 0.21 (males) to 0.33 (females). For DBP reactivity, heritability to the cold pressor test was higher (0.55) than that to mental stress even after including dominance variation (broad heritability of 0.29). Heritability estimates for HR reactivity to mental stress (0.43) and the cold pressor test (0.45) were very similar.

Formal testing revealed only mild heterogeneity in the twin correlation estimates across studies on mental stress, but confidence intervals around the pooled estimates for the twin correlations were fairly large and often included zero in the DZ groups. In view of the many ways in which a mental stress-testing experiment can be set up, even when using comparable stressors (task difficulty, amount of feedback, trial by trial reward/punishment, competing against a criterion or competition with visible scores of others, etc.) this

variation between studies in twin correlation estimates should be expected.

The major novel finding to arise from the meta-analysis was that the heritability of stress reactivity shows quantitative and qualitative sex differences. SBP reactivity to both mental stress and to the cold pressor test was more heritable in females than in males. In the studies reporting opposite sex twin pair correlations, these correlations were often comparable to the same-sex DZ twin pair correlations. A notable exception, however, was the very low DOS correlation for HR reactivity to the cold pressor test, suggesting that different genes affect this HR reactivity in males and females. Inspection of Tables 1 and 4 further suggest a possible decrease in heritability from adolescence to middle-age but we did not have sufficient data points to robustly test this hypothesis.

In the time frame of the mental stress tasks used (5–10 min) HR and BP reactivity are largely governed by the effects of the sympathetic and parasympathetic nervous system on cardiac output and vascular resistance. No study to date has addressed the heritability of cardiac output or peripheral resistance changes in response to stress. The latter is unfortunate because the patterning of vascular versus cardiac reactivity may be highly relevant to the type of disease outcome (Lawler et al., 2001; Sherwood and Turner, 1995). A few twin studies did test the effects of stress on parasympathetic nervous system reactivity, assessed as changes in heart rate variability in the respiratory frequency range or RSA (Grossman et al., 1990; Goedhart et al., 2007). In 208 middle-aged Dutch twin pairs RSA was measured during a rest period and a number of stress tasks (de Geus et al., 2007; Snieder et al., 1997). Heritability of the RSA decreases during a tone avoidance task was 0.24 and 0.33 in males and females, respectively (Snieder et al., 1997). However, no significant heritability was found for RSA decreases during an RT or MA task (de Geus et al., 2007). In 427 European American and 308 African American adolescent twins, Wang et al. (2009) measured RSA at rest and during three mental stressors. Heritability of the aggregated RSA decrease was 0.49. Significant heritability of aggregated RSA reactivity across two mental stressors was also found in 320 Dutch adolescent twins, albeit with a more modest heritability estimate of 0.09 (de Geus et al., 2007).

A limitation of most twin studies performed so far, and hence of the meta-analysis based on these studies, is that they analyzed reactivity as a change score. That way, the heritability estimates will reflect an inseparable mix of newly emerging genetic or environmental influences during stress and an amplification or dampening of genetic or environmental influences already present at rest. Emerging genes are genes that are truly expressed only during stress. They contribute to the heritability of a cardiovascular trait only when it is measured under stressful conditions. Amplified genes are genes that have an effect on individual differences in a cardiovascular trait at rest, but these effects become stronger under stress. As shown by de Geus et al. (2007) for RSA and DBP reactivity, significant amplification may occur even when change scores (reactivity) are not heritable. To explicitly test for emergence and amplification, bivariate analysis of resting and aggregated stress levels are needed and we reinforce the plea of de Geus et al. (2007) that future studies should use bi- or multivariate (in case of combined mental and physical stressors) designs.

Taken together, the results of our meta-analysis convincingly show that cardiovascular reactivity to an acute mental challenge or cold pressor test is substantially heritable. The obvious next question is which genes might be responsible for this heritability. A comprehensive list of potential pathways and candidate genes is given by Imumorin et al. (2005) and Table 7 lists the available studies to date that have tested the association of candidate genes with BP and HR reactivity to stress.

Table 7
Summary of studies testing genetic associations with stress reactivity of SBP, DBP and HR.

Investigators	Ethnicity and sample size Mean age ± SD(range)	Analysis methods	Stressors	Genes	SNPs	Reactivity in the genotype groups and significance of the test used by the investigators to compare the genotype groups (see under analysis methods)		
						SBP (mmHg)	DBP (mmHg)	HR (bpm)
Li et al. (2001)	332 Caucasians from 100 MZ and 66 DZ twin pairs Age: 30.1 ± 12.0	ANOVA testing an effect of genotype on the change scores	1. MA 2. CP	*ADRB2*	Arg16Gly	NS	MA 10.0 Arg/Arg 8.0 Arg/Gly 7.5 Gly/Gly ($p < 0.05$) CP 8.4 Arg/Arg 7.0 Arg/Gly 7.0 Gly/Gly ($p < 0.05$)	NS
					Gln27Glu	NS	NS	NS
					Thr164Ile	NS	NS	NS
					−47C>T	NS	NS	NS
McCaffery et al. (2002)	309 Caucasians including 101 MZ and 44 DZ twin pairs Age: 21.1 ± 2.8 (18–30)	Regression of the baseline-adjusted change scores on genotype	1. Stroop 2. MA	*ADRA1B*	Ile178Ile	NS	NS	NS
					Gly183Gly	NS	NS	NS
				ADRA2A	−1291C>G	NS	NS	NS
				ADRB1	Ser49Gly	NS	NS	NS
					Gly386Arg	NS	Stroop and MA: 7.0 Gly/Gly and Gly/Arg 4.8 Arg/Arg ($p < 0.01$)	NS
				ADRB2	Arg16Gly	NS	NS	NS
					Gln27Glu	NS	NS	NS

Table 7 (*Continued*)

Investigators	Ethnicity and sample size	Analysis methods	Stressors	Genes	SNPs	Reactivity in the genotype groups and significance of the test used by the investigators to compare the genotype groups (see under analysis methods)		
	Mean age±SD(range)					SBP (mmHg)	DBP (mmHg)	HR (bpm)
Liu et al. (2006)	47 Caucasians; Asian-American	ANOVA testing the interaction of genotype and condition (rest vs. task period)	Stroop	*ADRB2*	Arg16Gly	MAP: NS		NS
					Gln27Glu	MAP: NS		NS
	Hispanic/Latino							
	East Indian individuals: 23 males and 24 females Age: 21–49							
Poole et al. (2006)	228 African Americans and 222 Caucasians Age: 18.5 ± 2.7	MANOVA testing effect of haplotype on change scores with baseline levels as covariates	1. Video game 2. CP	*ADRB2*	Gly16Arg	NS	NS	n.a.
					Gln27Glu	NS	NS	n.a.
Hassan et al. (2008)	148 African American and Caucasian CAD patients: 103 males and 45 females Age: 64.0 ± 9.0	MANOVA testing an interaction of genotype and condition (rest vs. stress) with baseline level as a covariate	Public-speaking task	*ADRB1*	Ser49Gly	NS	NS	NS
					Gly389Arg	NS	NS	NS
				ADRB2	Gly16Arg	NS	NS	NS
					Gln27Glu	NS	NS	NS
					523C>A	NS	NS	NS
Kurnik et al. (2008)	40 African Americans and 39 Caucasians Age: 25.7 ± 5.3	Regression of the baseline-adjusted change scores on genotype	CP	*ADRA2C*	del322–325	28.6 del/del 18.8 del/ins and ins/ins ($p = 0.016$)	19.7 del/del 13.9 del/ins and ins/ins ($p = 0.058$)	30.9 del/del 13.4 del/ins and ins/ins ($p = 0.004$)
				GNB3	825C>T	NS	NS	20.4 T/T 12.1 C/T and C/C ($p = 0.003$)
Zhang et al. (2004)	294 Caucasians: 21 MZM, 8 DZM, 82 MZF, 30 DZF, and 7 DOS Age: 42.0 ± 1.0 (15–84)	SOLAR based regression of genotype on the change scores	CP	*TH*	Repeat polymorphism: two most common (TCAT)n alleles, (TCAT)6 and (TCAT)10	NS	NS	NS
Rao et al. (2008)	172 Caucasians: 119 MZ and 53 DZ	SOLAR based regression of genotype on the change scores	CP	*TH*	−824C>T	18.4 T/T	15.4 T/T	NS
	Age: 15–84					15.1 T/C 10.5 C/C	11.9 T/C 7.9 C/C	
					−801G>C	NS	NS	NS
					−581A>G	NS	NS	NS
					−494G>A	NS	NS	NS
Treiber et al. (2003a,b)	161 African Americans 213 Caucasians Age: 18.6 ± 2.7	ANCOVA testing an effect of genotype on change scores with baseline level as a covariate	1. Video game (VG) 2. Forehead cold	*ET-1*	Lys198Asn	VG in low SES subgroup 15.5 Asn/Asn; 13.1 Asn/Lys and Lys/Lys ($p < 0.05$)	VG in Obese subgroup 13.0 Asn/ASn 10.0 Asn/Lys and Lys/Lys[a] ($p_{Interaction} < 0.04$)	NS

Malhotra et al. (2004)	235 African Americans 262 Caucasians Age: 18.5±2.6	ANCOVA testing an effect of genotype on change scores with baseline level as a covariate	Video game	*NOS3*	Glu298Asp	NS	African Americans non-Obese 13.5 Glu/Glu 9.4 Asp/Glu and Asp/Asp ($p_{interaction} < 0.04$) European Americans, Obese 13.3 Glu/Glu 9.4 Asp/Glu and Asp/Asp ($p_{interaction} < 0.04$)	NS
Boomsma et al. (1991)	160 Caucasian twin pairs and their parents Age Twins: 16.7 (14–20) Fathers: 48.0 Mothers: 46.0	MANOVA using genotype by condition (rest, stressors)	1. RT 2. MA	*PI*	M, S/Z	Fathers, MA 12.8 MM 9.2 Non-MM (p=0.021)	NS	n.a.
Williams et al. (2001)	30 Caucasians 24 African Americans 36 males and 18 females Age: 18–49	ANOVA using genotype by condition (rest, stress)	Anger and sadness recall	*5-HTT*	short (*s*) and long (*l*) alleles	MAP 2.3 *s/s* 9.1 *s/l+l/l*[a] ($p < 0.0001$)		Effects not reported but significant at $p < 0.05$
McCaffery et al. (2003)	382 Caucasians 131 MZ and 60 DZ twin pairs Age: 21.0±2.8	ANOVA on genetically independent subset using genotype by task (Stroop, MA) on baseline-adjusted change scores	1. Stroop 2. MA	*5-HTT*	Short (*s*) and long (*l*) alleles	NS	NS	Females (MA+Stroop) 14.9 *s/s* 6.7 *l/s* 9.0 *l/l* ($p < 0.01$)
Williams et al. (2008)	94 AfricanAmericans and 71 Caucasians; 94 males and 71 females Age: 35.1 (18–50)	Regression of the baseline-adjusted change scores on genotype	Anger and sadness recall	*5-HTT*	Short (*s*) and long (*l*) alleles	11.4 *l/l* 9.9 *l/s* 7.2 *s/s* ($p < 0.047$)	8.6 *l/l* 7.2 *l/s* 4.3 *s/s* ($p < 0.0001$)	8.7 *l/l* 8.5 *l/s* 5.5 *s/s* ($p < 0.033$)

ADRB1: adrenergic receptor-β1; ADRB2: adrenergic receptor-β2; ADRA1B: adrenergic receptor-α1b; ADRA2A: adrenergic receptor-α2a; ADRA2C: adrenergic receptor-α2c; TH: tyrosine hydroxylase; ET-1: endothelin-1; NOS3: nitric oxide synthase 3; PI: protease inhibitor; 5-HTT: serotonin transporter; CP: cold pressor test; MA: mental arithmetic task; Stroop: color-word interference task; RT: reaction time task; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; MAP: Mean arterial pressure; TPR: total peripheral resistance; BMI: body mass index; CAD: coronary artery disease; SES: socioeconomic status; MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females; DOS: dizygotic opposite sex.

n.a.: data not given in the article.

NS: non-significant association.

[a] Effect sizes were extracted from figures in articles.

Heritable individual differences in BP and HR reactivity may arise from variation in genes that code for elements of the vagal and sympathoadrenal systems, including transmitter synthesis, release and reuptake, enzymatic degradation and receptor density and sensitivity. The β1- and β2-adrenergic receptors, for instance, play an important role in the cardiac response to neural and hormonal adrenergic stimulation as well as in the vasodilatory response (Brodde et al., 2006; Dishy et al., 2001). Non-synonymous variants in the genes coding for these receptors (*ADRB1* and *ADRB2*), i.e. variants that change an amino acid in the protein, have been associated with altered cardiac and vascular responses to various adrenergic agonists and are suspected to modulate cardiovascular disease risk (Brodde, 2008). In keeping, most association attempts have focused on variation in genes that code for these receptors.

In a study of healthy twins, higher DBP reactivity (+2.2 mmHg) was found in carriers of the Gly allele at position Gly386Arg of the *ADRB1* gene compared to Arg/Arg homozygotes (McCaffery et al., 2002). Although a study in cardiac patients failed to replicate this effect, patients that were homozygous for the Ser allele at position Ser49Gly in *ADRB1* gene were more likely to experience stress-induced myocardial ischemia (Hassan et al., 2008). Evidence to support a role for the *ADRB2* gene has been less compelling. In a study of Li et al. (2001) Arg/Arg homozygotes for the *ADRB2* Arg16Gly polymorphism had higher DBP reactivity to a mental arithmetic task (+2.5 mmHg) and a cold pressor test (+1.4 mmHg) than Gly/Gly homozygotes. Three other studies failed to replicate this finding (Liu et al., 2006; McCaffery et al., 2002; Poole et al., 2006) and no evidence was found for an effect of three other variants in this gene.

The most striking association result so far was found for the α_{2C}-adrenergic receptor (Kurnik et al., 2008). There is substantial evidence that α_{2C}-adrenergic mechanisms in the central nervous system affect the level of sympathetic drive to the heart and blood vessels. Noradrenergic activation of the α_{2C}-adrenergic receptor located on the presynaptic membrane acts to inhibit further release of noradrenaline from sympathetic nerves and adrenaline from the adrenal gland, whereas stimulation of the postsynaptic variant on vascular smooth muscle induces vasoconstriction. A common deletion of 12 base pairs that code for 4 amino acids (del322–325) in the *ADRA2C* gene causes a marked decrease in the response to adrenergic agonists (Small et al., 2000). Homozygotes for this deletion had higher HR (+17.5 bpm), SBP (9.8 mmHg) and DBP (+5.8 mmHg) reactivity to the cold pressor test than the combined heterozygote and insertion/insertion groups.

An additional SNP (C825T) in the gene coding the hetero-trimeric guanine nucleotide-binding protein B3-subunit (*GNB3*) was also significantly associated with HR reactivity. Homozygous T-allele carriers increased their HR on average 8.3 bpm more during stress than non-T carriers. This makes sense since G-proteins mediate intracellular signaling transduction of adrenergic receptors, including the α_{2C}-adrenergic receptor. Importantly, the strong ethic differences in HR reactivity, with black participants responding more strongly than white participants, largely disappeared when the analysis accounted for the higher frequency of the deleterious variants of these genetic variants in black participants (Kurnik et al., 2008). Genetic variation in other α-adrenergic receptor subtypes (*ADRA1B* and *ADRA2A*) has been tested also (McCaffery et al., 2002), but no such clear associations with stress reactivity emerged as for *ADRA2C*.

On the side of catecholamine synthesis, tyrosine hydroxylase (TH) has drawn most of the research attention. TH is the rate-limiting enzyme in the synthesis of the catecholamines. Testing a repeat polymorphism, Zhang et al. (2004) reported no effect of the number (TCAT) repeats on SBP, DBP and HR reactivity to the cold pressor test. Testing four SNPs in the promoter region of the gene in the same sample, however, revealed a significant positive association between the number of T alleles at base pair position −824 and SBP and DBP reactivity to the cold pressor test (Rao et al., 2008). Homozygous T-allele carriers had higher DBP (+7.6 mmHg) and SBP (+9.9 mmHg) than non-T carriers. Parallel effects were seen on catecholamine reactivity and the SNP accounted for 5.5% of the increases in plasma epinephrine and 1.5% of the increases in plasma norepinephrine.

A second system that responds fast enough to influence acute stress reactivity is the endothelial system that controls vascular smooth muscle function via production of vasoactive substances such as nitric oxide (NO), a potent vasodilator, and endothelin-1 (ET-1), a potent vasoconstrictor (Spieker et al., 2002). Researchers at the Medical College of Georgia tested the associations between non-synonymous SNPs in the ET-1 (Lys198Asn) and the NOS3 gene (Glu298Asp) and reactivity to a video game and forehead cold stimulus in a cross-sectional sample of African and European American normotensive young adults (Malhotra et al., 2004; Treiber et al., 2003a). Both polymorphisms showed associations with BP reactivity to the video game stressor in a complex pattern that depended upon obesity, ethnicity and SES. Among the obese, homozygote carriers of the ET-1 198 Asn allele had greater increases in DBP (+3.0 mmHg) than non-carriers. Homozygote Asn allele carriers who came from lower SES backgrounds exhibited higher SBP reactivity (+2.4 mmHg) than non-carriers. Carrier status for the NOS3 298Asp allele interacted with ethnicity and obesity status for diastolic BP reactivity such that in non-obese African Americans Glu/Glu homozygotes exhibited greater diastolic BP reactivity (+4.1 mmHg) compared to non-Glu allele carriers. Among obese European Americans, higher diastolic BP reactivity (+3.9 mmHg) was also found in the Glu/Glu homozygotes (Malhotra et al., 2004).

A further source of genetic differences in reactivity may be found in the central nervous system at the level of subjective perception of the stressor. Subjective feelings of threat are the core determinant of the generation of the autonomic responses underlying stress reactivity. Reactivity may be particularly sensitive to variation in serotonergic functioning, since selective serotonin reuptake inhibitors (SSRIs) that inhibit the serotonin transporter have been shown to reduce cardiovascular reactivity to mental stressors or emotion-inducing stimuli (Golding et al., 2005; Kemp and Nathan, 2004). Three studies have tested an association between a functional genetic variant in the linked polymorphic region (5-HTTLPR) of the serotonin transporter gene and cardiovascular stress reactivity. The results have been somewhat confusing, which parallels the fate of this genetic variant in psychiatric genetics at large (e.g. Caspi et al., 2003; Risch et al., 2009). Williams et al. (2001) reported that individuals carrying one or two long (*l*) alleles showed higher mean arterial pressure (MAP) reactivity (+6.8 mmHg) to a session of guided recall of moments of anger and sadness than s-allele homozygotes. This result was replicated and extended in a larger sample, where *l*-allele homozygotes had higher SBP (+4.2 mmHg), DBP (+4.3 mmHg), and HR (+3.2 bpm) reactivity compared to *s*-allele homozygotes (Williams et al., 2003). In sharp contrast, McCaffery et al. (2003) found a detrimental effect of the *s*-allele, particularly in females. Females with an *s/s* genotype exhibited aggregated HR reactivity across a Stroop and mental arithmetic task that was higher than that in males of the same genotype (+11.4 bpm) or females having either one (+8.2 bpm) or two long alleles (+5.9 bpm). In this study, no association between 5-HTTLPR genotype and SBP or DBP reactivity was found.

From the brief review above it is clear that, in spite of the significant heritability emerging from twin studies, independently and consistently replicated genetic variants that explain this heritability are still at large. This is not too surprising. As reactivity

is likely to be influenced by multiple genes and interactions of small effect, the effect size for each gene is generally expected to be small. Standard power calculations show that up to 1000 participants are required to detect gene main effects (http://hydra.usc.edu/gxe/). As reviewed by McCaffery et al. (2007) the required sample size will be even larger if one of the alleles is rare (e.g. less than 5–10%) or a large number of markers is typed and the statistical criterion, typically set at 0.05 for two-tailed tests, has to be adjusted for multiple comparisons. Also, since our results show that different genes may be expressed in men and women and potentially across age, samples may be required that are either homogeneous for gender and age or large enough to allow testing in subsamples. Finally, a number of association studies have tested genotype effects on task minus resting baseline change scores, sometimes even correcting for baseline levels. As with twin studies on reactivity, a bi- or multivariate approach that uses genotype and condition (e.g. rest, mental stress, physical stress, pharmacological challenge, etc.) as factors may be the optimal approach. The interaction between genotype and condition captures both emergence and amplification, whereas using change scores corrected for baseline levels may fail to detect genes that influence both resting levels and reactivity. Such genes do exist as was shown by Boomsma et al. (1991) for the α-1-antitrypsin (*ATT*) gene. The two rare deficiency alleles of a highly polymorphic locus in this gene reduced both absolute SBP levels (−7.0 mmHg) and the SBP reactivity (−3.6 mmHg) to mental stress among adult males. Correcting for the gene effects on the baseline would likely have removed the evidence for its effect on reactivity.

The concerns voiced above in no way disqualify the pioneering studies listed in Table 7, nor their at times encouraging results as for the common polymorphisms in the *TH* and *ADRA2C* genes. On the contrary, they aim to constitute a call for continued efforts in this area to provide independent replication of these studies. Sufficient efforts by multiple laboratories will allow future meta-analysis on their combined association results as a way to separate false positives from truly causal gene variants.

In summary, twin studies find strong evidence for a genetic contribution to individual differences in cardiovascular reactivity to stress, which is a biomarker for CVD. Future progress in genetic association studies, that include measures of sympathetic and vagal reactivity, may help uncover the molecular pathways from genes to stress reactivity. The long term aim is improved identification of at-risk subjects and timely person-specific intervention.

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CHAPTER 6

Genetic influence on blood pressure and underlying hemodynamics measured at rest and during stress

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Genetic Influence on Blood Pressure and Underlying Hemodynamics Measured at Rest and During Stress

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Objective: This study examined the genetic and environmental contributions to the individual differences in blood pressure (BP) levels and underlying hemodynamic characteristics at rest and during mental challenge tasks in a large twin cohort of youth. Including both European American and African American twins further allowed examination of potential ethnic differences. **Methods:** We studied cardiovascular reactivity to two stressors (car-driving simulation and a social stressor interview) in 308 European American and 223 African American twin pairs including monozygotic twin pairs and same-sex as well as opposite-sex dizygotic twin pairs (mean [standard deviation] age = 14.7 [3.1]). Variables included systolic and diastolic BP, heart rate, stroke volume, cardiac output, and total peripheral resistance. **Results:** Heritability indices for levels at rest and during stress were high (31%–73%) and comparable between ethnic groups. A common genetic factor accounted for both resting and stress levels explaining 23% to 58% of the total variance. The increases in heritability indices for BP and heart rate from rest to stress are mostly explained by newly emerging genetic influences on the added stress component. Indices for hemodynamic variables remained stable from rest to stress owing to a simultaneous decrease in genetic and environmental variances. **Conclusions:** Cardiovascular measures obtained during rest and stress show substantial heritability that is comparable between individuals of African and European descent. Most of the variance in both resting and stress levels is explained by common genetic factors, although other genetic factors that only contribute to cardiovascular levels during stress are also important. **Key Words:** blood pressure, hemodynamics, reactivity, heritability, ethnicity, twin.

AA = African American; **A or a^2** = additive genetic effects; **BP** = blood pressure; **C or c^2** = shared environmental effects; **CO** = cardiac output; **CV** = cardiovascular; **CVD** = cardiovascular disease; **CVR** = cardiovascular reactivity; **DBP** = diastolic blood pressure; **E or e^2** = nonshared environmental effects; **EA** = European American; **GEE** = generalized estimating equation; **h^2** = heritability; **HR** = heart rate; **SBP** = systolic blood pressure; **SV** = stroke volume; **TPR** = total peripheral resistance.

INTRODUCTION

Cardiovascular reactivity (CVR), defined as the magnitude or pattern of an individual's hemodynamic responses to behavioral stressors, has been identified as potentially playing a role in the development of coronary artery disease and hypertension (1). Large stress-induced blood pressure (BP) or heart rate (HR) elevations are hypothesized to lead, over time, to elevation of the tonic BP level and the development of coronary artery disease (2).

BP measured under certain standardized environmental challenges such as mental or physical stress may be more heritable than its unchallenged counterpart, potentially offering important advantages for gene-finding studies (3). However, a downside of expressing CVR as a change score is that its heritability reflects an inseparable mixture of genetic and environmental influences already present at rest with those newly emerging during stress. These influences can only be separated if both resting and challenged *levels* (as opposed to a change score) are analyzed in a bivariate model, that is, a model including two dependent variables. De Geus et al. (4) recently used such an approach to investigate BP during a stress challenge and test for the existence of gene-by-stress interaction within the context of a classic twin study. Genetic factors significantly contributed to individual differences in resting systolic BP (SBP) and diastolic BP (DBP) in the adolescent and middle-aged twin cohorts of Northern European ancestry included in this study. The effect of these genetic factors was amplified by stress for both SBP and DBP in the adolescent cohort and for SBP in the middle-aged cohort. In addition, stress-specific genetic variation emerged for SBP in the adolescent cohort. It was concluded that exposure to stress may uncover new genetic variance and amplify the effect of genes that already influence the resting level.

The aim of the current study was to replicate the work by de Geus et al. (4) in adolescents and extend it in two important ways. First, we extended it to another ethnic group by including not only European American (EA) but also a group of African American (AA) twins. Second, we extended it to include cardiac output (CO) and total peripheral resistance (TPR) of the systemic vasculature as hemodynamic determinants of BP, because a given increase in BP can be the result of an increase in CO, an increase in TPR, or a combination of alterations in both parameters (5).

The goal of this study, therefore, was to estimate the contribution of genes and environment to the individual differences in levels of BP and underlying hemodynamic characteristics at rest and during stress using a bivariate approach in a large twin cohort of youth. Unlike previous studies, inclusion of both EA and AA twins further allowed examination of potential ethnic differences.

METHODS

Participants

The present study comprised participants from the Georgia Cardiovascular Twin Study, which was established in 1996 (6,7). Participants were 308 EA and

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226 ΛΛ twin pairs from the southeastern United States, including pairs of the same and the opposite sex (mean [standard deviation] age = 14.7 [3.1] years; range, 10.0–25.9 years). See Table 2 for the number of pairs for each sex by zygosity group in EAs and ΛΛs. Zygosity was determined using five standard microsatellite markers in DNA collected with buccal swabs (8). Recruitment of twin pairs into the Georgia Cardiovascular Twin Study has been described previously (7), as have been the criteria to classify participants as ΛΛ or EΛ (9). The study was approved by the institutional review board, and all participants (or parents if participants were <18 years) provided written informed consent.

All twin pairs were reared together and were apparently healthy based on (parental) report of the child's medical history. Three twin pairs were excluded because one twin of each pair had an SBP level higher than 160 mm Hg or a DBP level higher than 90 mm Hg (10). None of the participants used any antihypertensive medication.

Measurements

All participants were asked to refrain from tobacco use and drinking alcohol for 11 hours before the visit. After arrival in the laboratory, anthropometric data were collected using established protocols (11). As a measure of general obesity, body mass index (BMI) was computed as weight/height^2. Participants were instrumented for the recording of SBP, DBP, and mean arterial pressure by using a Dinamap 1864 SX (Critikon Incorporated, Tampa, FL) and HR by electrocardiograph. In addition, stroke volume (SV) was measured by bioimpedance cardiography (NCCOM; BoMed Medical Manufacturing Ltd., Irvine, CA). The NCCOM yields reliable and valid measures of CO when compared with simultaneous thermodilution and Fick-derived measures of CO obtained from supine individuals (12,13). CO (SV * HR) was indexed by body surface area (i.e., cardiac index). TPR index was calculated as mean arterial pressure/cardiac index. Bioimpedance measures were not available for eight participants because of equipment failure.

Baseline hemodynamics were calculated based on the average of minutes 11, 13, and 15 while participants lay (supine) on a hospital bed. After the resting evaluation period, the participants engaged in the virtual-reality car-driving simulation test (5 minutes) and the social stressor interview (10 minutes) using a standardized protocol during which hemodynamics were recorded and BP was measured every 2 minutes. The two stressors have been successfully used in our laboratory studies for more than 10 years (14–16).

The virtual-reality car-driving stressor. Briefly, the participant wore a Kaiser-Optic Visual Immersion Monitor (VIM 500) fitted on his/her head. The VIM 500 was interfaced with a Panasonic Real 3DO Interactive Multiplayer System. The participant played "Need for Speed" under the condition of challenge (i.e., money incentive) without harassment for 5 minutes.

The social competence interview was administered using an established protocol (17). Briefly, participants discussed a recent interpersonal interaction, which resulted in significant anger and/or frustration. A 10-minute structured interview was used to guide the participant in describing the event, including his/her affective and behavioral responses and summarization of outcome of the event.

Analytical Approach

Previous studies have shown increased reliability of interindividual differences in the response to stress when multiple stressors are aggregated to a single stress level (18). In the present study, we summed the levels of BP and hemodynamics across all observations for each stressor and then averaged the values for both of the stressors. This result yielded a single score for the mean stress level for each parameter. CV reactivity (change score) was calculated as average stress level minus resting level.

Genetic Modeling of Twin Data

Univariate analysis for each dependent variable separately was firstly used to estimate the relative influence of genetic and environmental factors on individual differences of BP and underlying hemodynamic characteristics at rest and during stress. Sex differences in (co)variance were examined by comparing the full model, in which parameter estimates are allowed to differ in magnitude between male and female participants, with a reduced model in which parameter estimates are constrained to be equal across the sexes. In addition to those models, a scalar model was tested. In a scalar model, heritabilities are constrained to be equal across sexes, but total variances may be different (19).

Subsequently, we used a bivariate analysis of rest and stress levels corresponding to the path diagram shown in Figure 1. This path diagram depicts the typical structural equation modeling approach to twin resemblances, which has been described previously (20,21). In this approach, the variance in the observed traits (e.g., SBP at rest and SBP during stress) is decomposed into latent additive genetic, shared environmental, and unique environmental components. The effect of Λ represents the relative contribution of genetic variance to the total variance (heritability) in SBP at rest calculated as $a_{11}^2/(a_{11}^2 + c_{11}^2 + e_{11}^2)$.

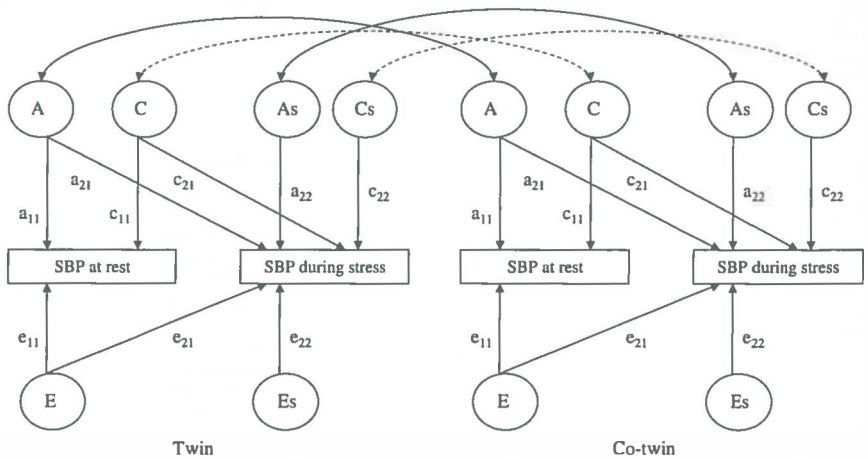

Figure 1. Bivariate twin model for genetic and environmental influences on systolic blood pressure (SBP). Biometrical genetic theory specifies that the additive genetic factors (denoted by Λ and As) of monozygotic twins are perfectly correlated (1.0), whereas those of dizygotic twins are correlated (0.5). Common environmental factors shared by twins from the same family (denoted by C and Cs) are correlated unity for both types of twins, whereas the unique environmental influences (E and Es) are always uncorrelated. Path coefficient a_{11} quantifies the effect of genetic influence Λ on SBP at rest, a_{21} quantifies the effect of Λ on SBP during stress, and a_{22} quantifies the effect of emergent genes in As on SBP during stress. In a similar way, path coefficients e_{11}, c_{11}, e_{21}, and c_{21} quantify the effects of common and unique environmental influences E and C on SBP at rest and during stress. e_{22} and c_{22} quantify the effect of emergent environmental influences in Es and Cs on SBP during stress.

TABLE 1. General Characteristics and Hemodynamic Measures at Rest and During Stress of European and African American Male and Female Participants

Characteristic	European Americans		African Americans		Ethnicity and Sex Effects[a]	
	Male	Female	Male	Female	Ethnicity, *p*	Sex, *p*
Participants, *n*	294	322	202	244		
Age, y	14.8 (2.9)	14.9 (3.1)	14.2 (2.7)	14.9 (3.5)	NS	NS
Height, m	1.64 (0.14)	1.58 (0.97)	1.63 (0.13)	1.59 (0.77)	NS	<.001
Weight, kg	59.1 (19.5)	54.5 (15.9)	60.3 (22.0)	58.1 (16.7)	NS	<.05
BMI, kg/m^2	21.5 (4.8)	21.5 (5.0)	22.2 (5.8)	22.9 (5.6)	NS	NS
Cardiovascular measures						
SBP at rest, mm Hg	110.1 (9.3)	105.7 (8.2)	112.9 (10.8)	110.2 (10.2)	<.001	<.001
SBP during stress[b], mm Hg	124.1 (12.8)	117.1 (10.8)	124.1 (13.0)	118.6 (11.5)	NS	<.001
SBP change score[c], mm Hg	14.0 (8.32)	11.3 (7.37)	11.2 (8.36)	8.33 (8.06)	<.001	<.001
DBP at rest, mm Hg	56.2 (5.8)	57.6 (5.4)	59.5 (5.9)	61.0 (7.0)	<.001	<.01
DBP during stress, mm Hg	68.4 (7.01)	69.8 (6.25)	70.4 (7.44)	70.7 (7.41)	<.01	<.05
DBP change score, mm Hg	12.1 (5.540	12.3 (5.20)	10.9 (5.44)	9.69 (5.23)	<.05	NS
HR at rest, beats/min	67.2 (11.7)	71.9 (11.7)	65.8 (10.9)	70.7 (10.9)	NS	<.001
HR during stress, beats/min	77.0 (12.5)	82.7 (12.0)	73.1 (11.1)	79.6 (11.6)	<.01	<.001
HR change score, beats/min	9.25 (6.13)	9.95 (6.38)	6.86 (5.48)	7.71 (6.38)	<.001	NS
SV at rest, ml/beat	87.1 (20.1)	88.9 (19.3)	83.3 (20.1)	85.2 (18.4)	NS	NS
SV during stress, ml/beat	76.2 (18.3)	79.1 (17.3)	75.5 (18.3)	77.5 (16.6)	NS	<.05
SV change score, ml/beat	−11.0 (8.88)	−9.82 (8.62)	−8.22 (8.43)	−7.66 (8.89)	<.01	NS
Cardiac index at rest, $l\ min^{-1}\ m^{-2}$	3.62 (0.78)	4.17 (0.78)	3.39 (0.69)	3.87 (0.83)	<.01	<.001
Cardiac index during stress, $l\ min^{-1}\ m^{-2}$	3.59 (0.77)	4.21 (0.75)	3.35 (0.62)	3.87 (0.79)	<.001	<.001
Cardiac index change score, $l\ min^{-1}\ m^{-2}$	−0.05 (0.38)	0.03 (0.48)	−0.05 (0.35)	0.01 (0.44)	NS	<.05
TPR index at rest, $mm\ Hg/l\ min^{-1}\ m^{-2}$	21.5 (5.20)	18.3 (3.95)	23.9 (5.47)	21.1 (5.60)	<.001	<.001
TPR index during stress, $mm\ Hg/l\ min^{-1}\ m^{-2}$	25.6 (6.11)	21.1 (4.10)	27.3 (5.56)	23.6 (5.49)	<.001	<.001
TPR index change score, $mm\ Hg/l\ min^{-1}\ m^{-2}$	4.08 (3.23)	2.81 (2.73)	3.55 (3.50)	2.33 (3.19)	NS	<.001

NS = not significant; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SV = stroke volume; TPR = total peripheral resistance.

Data are mean (SD), unless stated otherwise.

[a] Ethnic and sex effects on mean values were tested by generalized estimating equations in regression models that included age as a covariate in addition to ethnicity, sex, and their interaction; ethnic and sex interactions on mean values were not significant. Cardiac index = cardiac output/body surface area; TPR index = mean arterial pressure/cardiac index.

[b] Stress levels were aggregated values of two stress tasks.

[c] Change score = aggregated stress level − level at rest.

The heritability of SBP during stress is the summed effect of A and As and is calculated as $(a_{21}^2 + a_{22}^2)/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. We can further test whether the genes influencing SBP at rest are the same (i.e., $a_{22} = 0$?), partly the same (i.e., $a_{21} \neq 0$ and $a_{22} \neq 0$?), or entirely different (i.e., $a_{21} = 0$?) from SBP during stress. If they are partly the same, this bivariate model allows further determination of the amount of overlap between genes influencing SBP at rest and during stress by calculating the genetic correlation between the two traits $[r_g = COV_A(\text{trait 1, trait 2})/\sqrt{(V_A\text{trait1} * V_A\text{trait2})}]$. Shared and unique environmental correlations can be calculated in a similar fashion (19,22).

When going from rest to stress, the effects of the genetic differences between participants may be amplified ($a_{21} > a_{11}$) or dampened ($a_{21} < a_{11}$) by the stressors. In addition, entirely new genetic variation between participants may emerge only during stress, depicted by factor As. In this case, the path-coefficient a_{22} will differ significantly from zero ($a_{22} > 0$). This part of the total heritability of the stress level represents the influence of novel genetic effects only expressed during stress and is equal to $a_{22}^2/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. Both amplification/dampening and emergence effectively constitute forms of gene-by-stress interaction (23). For comparison with previous studies, we also computed heritability of reactivity as a change score, as described previously (4).

Model Fitting Procedure

All quantitative genetic modeling approaches were carried out separately for EAs and AAs. Before genetic analysis, SBP, DBP, and TPR index were log transformed to obtain better approximations of normal distributions. Effects of age, sex, ethnicity, and BMI were regressed out for all variables, and the residuals were used in model fitting and the calculation of twin correlations. Models were fitted to the raw data using normal theory maximum likelihood, allowing inclusion of incomplete data. For genetic modeling, a series of submodels nested within the full parameter ACE triangular (Cholesky) model were fitted to the multivariate variance-covariance matrices (a model including dominance genetic effects was not considered based on inspection of the twin correlations). The significance of variance components A, C, and E was assessed by testing the deterioration in model fit after each component was dropped from the full model. Emergence of new genetic factors was tested by a submodel that constrains the a_{22} parameter to zero. Amplification (or dampening) of genetic factors was tested by a submodel that constrains a_{21} and a_{11} to be equal.

Standard hierarchic χ^2 tests were used to select the best-fitting models in combination with Akaike information criterion ($=\chi^2 - 2df$). The model with

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TABLE 2. Twin Correlations for Each Sex by Zygosity Group in European and African Americans

	European Americans					African Americans				
Measure	MZM	DZM	MZF	DZF	DOS	MZM	DZM	MZF	DZF	DOS
Pairs, *n*	73	35	89	33	78	50	26	58	39	50
SBP										
Rest	0.59	0.48	0.58	0.36	0.10	0.69	0.30	0.50	0.35	0.14
Stress	0.78	0.38	0.71	0.45	0.05	0.86	0.31	0.67	0.38	0.05
Reactivity	0.60	0.35	0.41	0.39	0.13	0.59	0.18	0.52	0.38	0.08
DBP										
Rest	0.47	−0.07	0.49	0.27	0.25	0.64	0.36	0.54	0.17	0.30
Stress	0.70	0.31	0.64	0.39	0.18	0.75	0.30	0.74	0.47	0.25
Reactivity	0.54	0.29	0.47	0.22	0.05	0.49	0.27	0.57	0.36	−0.16
HR										
Rest	0.67	0.38	0.70	0.42	0.34	0.70	−0.02	0.66	0.35	0.39
Stress	0.81	0.40	0.67	0.38	0.36	0.71	0.22	0.70	0.24	0.48
Reactivity	0.42	0.23	0.39	0.22	0.15	0.35	0.33	0.38	0.05	−0.08
SV										
Rest	0.50	0.41	0.56	0.24	0.29	0.62	0.34	0.54	0.57	0.02
Stress	0.51	0.31	0.63	0.06	0.29	0.59	0.44	0.52	0.62	0.06
Reactivity	0.18	0.12	0.19	0.14	0.03	0.21	0.03	0.14	0.60	0.24
Cardiac index										
Rest	0.60	0.45	0.45	0.55	0.27	0.48	0.05	0.31	0.26	0.03
Stress	0.55	0.47	0.43	0.28	0.16	0.31	0.16	0.29	0.24	0.07
Reactivity	0.06	0.23	0.21	0.03	0.01	0.45	0.20	0.22	0.29	0.18
TPR index										
Rest	0.49	0.43	0.46	0.58	0.29	0.55	0.02	0.40	0.29	0.09
Stress	0.57	0.55	0.51	0.20	0.17	0.43	0.15	0.31	0.35	0.12
Reactivity	0.25	0.50	0.21	−0.14	0.04	0.21	0.12	0.15	0.39	0.23

MZM = monozygotic males; DZM = dizygotic males; MZF = monozygotic females; DZF = dizygotic females; DOS = dizygotic opposite sex; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SV = stroke volume; TPR = total peripheral resistance.
Residuals were used after effects of age, sex, ethnicity, and body mass index were regressed out for all measures.

the lowest Akaike information criterion reflects the best balance of goodness of fit and parsimony.

Statistical Software

Ethnic and sex effects on mean values were tested by generalized estimating equations (GEEs) in regression models that included age as a covariate in addition to ethnicity, sex, and their interaction. GEE takes the nonindependence between twins into account and yields unbiased standard errors and *p* values (24). Data handling, preliminary analyses, and GEEs were performed with STATA software (StataCorp., College Station, TX). Quantitative genetic modeling was performed with Mx software, a computer program specifically designed for the analysis of twin and family data (25).

RESULTS

Table 1 shows mean values of general characteristics and CV reactivity measures for EA and AA male and female participants in the twin sample. The mean age of the sample was 14.7 years (range, 10.0–25.9 years). As shown in Table 1, age, height, weight, and BMI were very similar for AAs and EAs. Male participants were taller and heavier than female participants. At rest, AAs showed significantly higher SBP, DBP, and TPR index levels but lower cardiac index than EAs. Female participants had higher DBP, HR, and cardiac index but lower SBP and TPR index levels than male participants. During stress, EAs showed significantly higher cardiac index and HR but lower DBP and TPR index levels than AAs. Male participants had higher SBP and TPR index but lower DBP, HR, SV, and cardiac index than female participants. EAs showed significantly higher SBP, DBP, HR, and SV reactivity to stress compared with AAs. Male participants showed higher SBP, cardiac index, and TPR index reactivity to stress than female participants. There was no significant interaction between ethnicity and sex on any of the measures.

Table 2 shows the twin correlations for each sex, by zygosity group in EAs and AAs. For rest and stress levels in both EAs and AAs, monozygotic correlations showed consistently higher values than did dizygotic correlations, and mostly, the monozygotic correlations exceeded dizygotic correlations by almost half, suggesting genetic factors as the main source of familial resemblance in these traits. Correlational patterns for hemodynamics change scores were less clear-cut compared with those for levels, which highlights the advantage of modeling all information available in the resting and stress

TABLE 3. Bivariate Heritability Estimates in European and African Americans

	Rest Level, h^2 (95% CI)	Stress Level, h^2 (95% CI)	Amplification or Dampening of Genes Acting on Resting Level	Specific h^2 Caused By Genes Emerging During Stress (95% CI)	Reactivity[a], h^2 (95% CI)
European Americans					
SBP	0.61 (0.52–0.68)	0.68 (0.60–0.75)	No, $a_{21}ca_{11} = 1.19$	0.25 (0.19–0.31)	0.48 (0.37–0.57)
DBP	0.40 (0.30–0.49)	0.67 (0.58–0.73)	No, $a_{21}/a_{11} = 0.85$	0.28 (0.20–0.35)	0.38 (0.27–0.47)
HR	0.66 (0.58–0.72)	0.70 (0.64–0.76)	No, $a_{21}/a_{11} = 1.01$	0.12 (0.08–0.16)	0.42 (0.29–0.53)
SV	0.51 (0.40–0.60)	0.53 (0.42–0.62)	Yes, $a_{21}/a_{11} = 0.86$	NS	NS
Cardiac index	0.49 (0.39–0.58)	0.51 (0.41–0.59)	No, $a_{21}/a_{11} = 0.89$	NS	NS
TPR index	0.49 (0.40–0.58)	0.55 (0.46–0.64)	No, $a_{21}/a_{11} = 0.96$	0.08 (0.04–0.13)	0.21 (0.09–0.33)
African Americans					
SBP	0.60 (0.49–0.68)	0.72 (0.63–0.79)	No, $a_{21}/a_{11} = 1.07$	0.24 (0.17–0.31)	0.50 (0.37–0.60)
DBP	0.53 (0.42–0.62)	0.73 (0.65–0.80)	No, $a_{21}/a_{11} = 0.91$	0.22 (0.14–0.29)	0.37 (0.24–0.50)
HR	0.66 (0.56–0.74)	0.68 (0.58–0.75)	No, $a_{21}/a_{11} = 1.04$	0.10 (0.05–0.15)	0.34 (0.18–0.48)
SV	0.58 (0.46–0.68)	0.58 (0.45–0.67)	Yes, $a_{21}/a_{11} = 0.84$	0.06 (0.02–0.10)	0.28 (0.11–0.42)
Cardiac index	0.38 (0.22–0.51)	0.31 (0.14–0.45)	Yes, $a_{21}/a_{11} = 0.70$	0.08 (0.03–0.12)	0.35 (0.20–0.49)
TPR index	0.44 (0.29–0.57)	0.39 (0.23–0.52)	Yes, $a_{21}/a_{11} = 0.77$	0.10 (0.05–0.16)	0.34 (0.18–0.48)

h^2 = heritability; CI = confidence interval; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SV = stroke volume; TPR = total peripheral resistance.

Residuals were used after effects of age, sex, ethnicity and BMI were regressed out for all measures.

[a] Reactivity was defined as change score calculated as average stress level minus resting level. Based on parameter estimates of the best-fitting bivariate models, the heritability of reactivity can be derived as $((a_{21} - a_{11})^2 + a_{22}^2)/((a_{21} - a_{11})^2 + a_{22}^2 + (c_{21} - c_{11})^2 + c_{22}^2 + (e_{21} - e_{11})^2 + e_{22}^2)$.

levels using a bivariate approach rather than modeling the change scores.

We first used univariate models to estimate heritabilities and to test potential sex effects (data not shown). The best-fitting models included additive genetic and unique environmental components (AE models) for all measures, except for cardiac and TPR index at rest in EAs and SV and TPR index change score in AAs, in which models including common and unique environmental components (CE models) were shown to have the best fit. In EAs, scalar sex effects were found in cardiac index change score, with female participants showing larger total variability than male participants, and in all TPR index variables, with male participants showing larger total variability than female participants but equal heritability across sexes. In AAs, scalar effects were detected in both resting and stress levels of SV, all cardiac index variables, and resting levels for TPR index, with female participants showing larger total variability than males, whereas for TPR index change score, male participants showed larger total variability than did female participants. Thus, a number of variables showed differences in total variances between male and female participants, but no significant sex differences in genetic or environmental parameter estimates were observed. On the basis of these results, we equalized total variances of SV, cardiac index, and TPR index in male and female participants before bivariate modeling and estimated all parameters by combining data from male and female participants in these models.

Results from bivariate testing, using the model depicted in Figure 1, are shown in Tables 3 and 4 and Figures 2 and 3. Models including only an additive genetic and unique environmental component (AE models) gave the best overall fit to the data for all six traits in both EAs and AAs. That is, significant heritabilities were found for resting and stress levels for all variables in both ethnic groups. The heritability estimates were highly comparable across EA and AA participants. As can be judged from the overlap of 95% confidence intervals, neither for resting nor for stress levels were any ethnic effects on heritability observed.

A common genetic factor was found to influence both resting and stress levels for all variables. This factor represents genes that act on both resting and stress levels and corresponds to factor A in Figure 1. As shown in Figure 2, 39% to 52% of the total variance of stress BP could be attributed to genes that also influenced resting BP. A somewhat larger common genetic factor was found for HR (58%) in both EAs and AAs. For SV and cardiac index in EAs, the heritability of stress levels could entirely be explained by genetic factors that also influenced resting level (53% and 51%). Forty-eight percent and 28% of the total variance in EAs and AAs, respectively, were attributed to genes that also influenced resting TPR index (Fig. 3).

The effect of this common genetic factor was dampened for SV in both EAs and AAs, and for cardiac and TPR index in AAs only. Furthermore, new genetic factors corresponding to factor As in Figure 1 emerged for SBP, DBP, HR, and TPR index in EAs and all variables in AAs (Table 3). These factors accounted for 6% to 28% of the total variance of these variables during stress. Comparing this with the total heritability of the stress levels, which varied from 31% to 73%, shows that this emergent genetic factor accounts for a smaller part of the total variance during stress than the effect of the common genetic factor also acting on the resting level. Regarding the reactivity, the heritability of change scores varied from 21% to 50%, except

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TABLE 4. Phenotypic (r_P), Genetic (r_A), and Environmental (r_E) Correlations Between Rest and Stress Levels as Well as the Proportion of r_P Explained by Genetic (A) or Environmental (E) Factors Based on the Best-Fitting Bivariate Models

	Phenotypic Correlation		Additive Genetic Correlation		Unique Environmental Correlation		Proportions of r_P
	r_P	95% CI	r_A	95% CI	r_E	95% CI	A/E[a]
European Americans							
SBP	0.73	0.69–0.77	0.86	0.79–0.91	0.52	0.41–0.62	0.75/0.25
DBP	0.62	0.57–0.67	0.71	0.60–0.81	0.54	0.43–0.63	0.60/0.40
HR	0.85	0.83–0.87	0.91	0.88–0.95	0.72	0.65–0.78	0.73/0.27
SV	0.86	0.84–0.88	0.96	0.92–1.00	0.75	0.68–0.80	0.58/0.42
Cardiac index	0.83	0.81–0.86	0.95	0.91–1.00	0.71	0.64–0.77	0.57/0.43
TPR index	0.80	0.77–0.83	0.92	0.86–0.96	0.68	0.60–0.75	0.59/0.41
African Americans							
SBP	0.74	0.69–0.79	0.84	0.76–0.90	0.59	0.47–0.69	0.74/0.26
DBP	0.70	0.65–0.75	0.81	0.72–0.89	0.53	0.39–0.65	0.73/0.27
HR	0.85	0.82–0.88	0.93	0.89–0.97	0.69	0.59–0.77	0.73/0.27
SV	0.88	0.85–0.90	0.95	0.90–0.98	0.78	0.71–0.84	0.63/0.37
Cardiac index	0.85	0.83–0.88	0.86	0.73–0.94	0.85	0.80–0.89	0.35/0.65
TPR index	0.82	0.78–0.85	0.85	0.74–0.93	0.79	0.72–0.85	0.43/0.57

A = additive genetic factor; E = unique environmental factor; CI = confidence interval; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SV = stroke volume; TPR = total peripheral resistance.
Residuals were used after effects of age, sex, ethnicity, and body mass index were regressed out for all measures.
[a] A/E is the percentage of the phenotypic correlation that is caused by genes (A) or environment (E), calculated from the following equation: $r_P = \left(\sqrt{h^2_{REST}} * r_A * \sqrt{h^2_{STRESS}}\right) + \left(\sqrt{e^2_{REST}} * r_E * \sqrt{e^2_{STRESS}}\right)$.

for SV and cardiac index in EAs, which showed no significant heritability for the change scores (Table 3).

When estimating to what extent phenotypic correlations between rest and stress levels can be explained by genetic or environmental factors that influence variables both at rest and during stress, genetic correlations were substantial, whereas unique environmental correlations were somewhat weaker but still highly significant (Table 4).

Compared with the rest condition, the total variance during stress increased for SBP, DBP, and HR in both EAs and AAs but, with the single exception of TPR index in EAs, decreased for all others. The increase in total variance for SBP, DBP, and

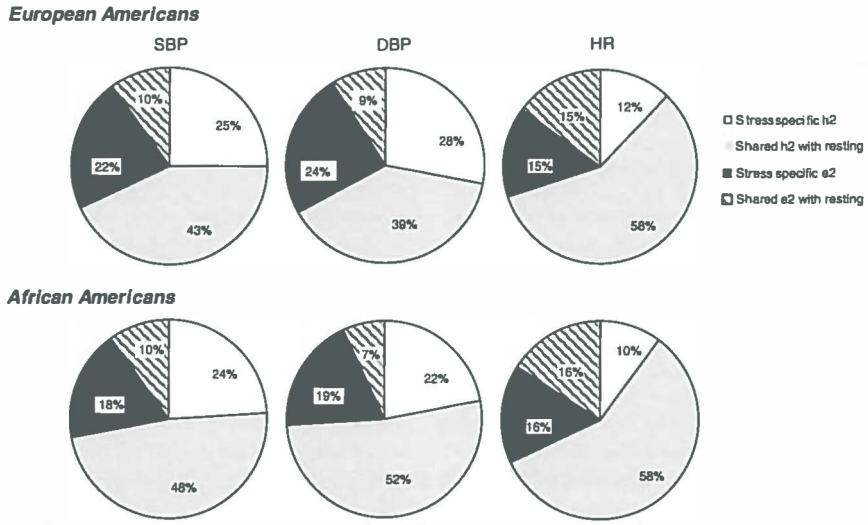

Figure 2. Sources of variance in stress SBP, DBP, and HR in comparison with resting SBP, DBP, and HR in EAs and AAs. SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate.

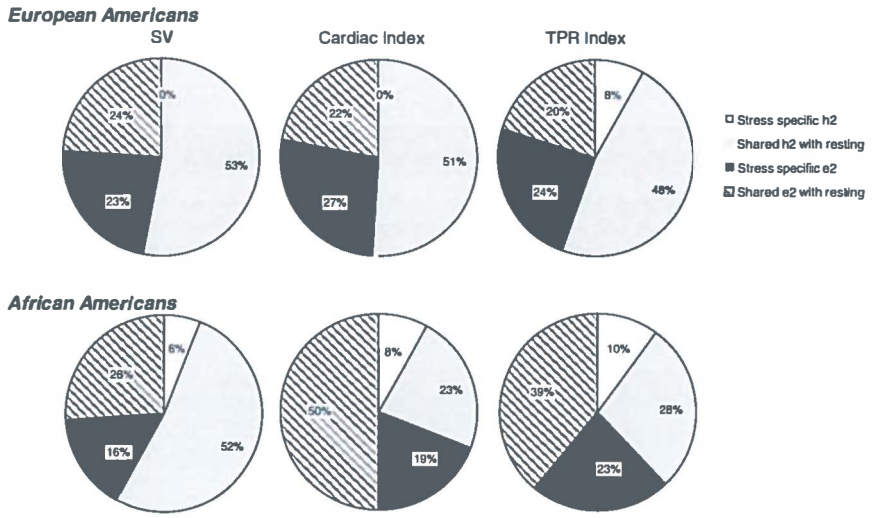

Figure 3. Sources of variance in stress SV, cardiac index, and TPR index in comparison with resting SV, cardiac index, and TPR index in EAs and AAs. SV = stroke volume; TPR = total peripheral resistance.

HR in both ethnic groups was mostly caused by an increase in the genetic variance, whereas environmental variance decreased or stayed about the same (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A68). Together with the results from Table 3, this confirms that increases in heritabilities of BP and HR from rest to stress in both EAs and AAs are mostly caused by newly emerging genetic influences during stress. For the hemodynamic parameters, heritabilities stayed fairly stable from rest to stress mostly caused by simultaneous decreases in genetic and environmental variances (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A68).

DISCUSSION

The intents of this study were to estimate the contribution of genes and environment to the individual differences in levels of BP and underlying hemodynamics at rest and during stress and to examine ethnic differences in a large sample of young twins. The results were in line with previous univariate analyses for resting levels of cardiovascular variables (10). The current bivariate analysis shows that results are comparable across ethnicity groups not only for rest as shown before but also for stress. Most genetic variance was explained by a common genetic factor influencing both resting and stress levels. Increases in heritabilities of BP and HR from rest to stress in both EAs and AAs were mostly caused by newly emerging genetic influences, whereas the heritabilities of hemodynamics stayed fairly stable from rest to stress owing to simultaneous decreases in genetic and environmental variances. CVR heritability varied from 21% to 50%, except for SV and cardiac index in EAs, which showed no significant heritability.

Previous studies of BP and HR reactivity simply used univariate models to estimate heritabilities of change scores (i.e., stress minus rest levels) as an inherent test of gene-stress interaction. We recently used meta-analysis to summarize results of all available twin studies that measured such BP and HR reactivity to mental stress in white participants (26). The pooled heritability estimate for change in HR was 43% with no effects of sex, very similar to our estimate of 42% in the current study. However, the present study showed higher heritabilities for SBP (0.48) and DBP reactivity (0.38) than the meta-analysis of Wu et al. (26), which estimated SBP reactivity at 0.26 and 0.38 for male and female participants, respectively, and at 0.29 for DBP reactivity. A major difference between the current and previous studies is that estimates for CVR heritabilities were derived from the best-fitting bivariate model of rest and stress levels. This has the important advantage that all available information in the bivariate variance/covariance matrices is used, providing more power to select the best-fitting model (27). Interestingly, our study is the first to present heritability estimates of BP and HR reactivity in AAs, which were found to be comparable with those in EAs.

The limitation of the studies using univariate analysis of change scores is that heritability estimates will reflect an inseparable mix of newly emerging genetic or environmental influences during stress and an amplification or dampening of genetic or environmental influences already present at rest (4). To explicitly test for emergence and amplification/dampening, we used bivariate analysis of resting and aggregated stress levels. We found that a single genetic factor (or set of genes) influenced both resting and stress levels for all variables. However, we also observed emergence of new genes under stress conditions for BP, HR, all hemodynamic variables in AAs, and TPR index in EAs. In line with these findings, using the same Georgia Cardiovascular Twin cohort measured 4 years later, Wang et al. (28) found

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that substantial overlap exists between genes that influence BP measured in the office, under laboratory stress and during real life, but that significant genetic components specific to each BP measurement also exist. These findings confirm that, partly, different genes or sets of genes contribute to BP regulation under different conditions. The study by de Geus et al. (4) in a Dutch adolescent and middle-aged twin cohort is the only other study that used bivariate analyses of rest and stress levels of BP and HR. Apart from being, on average, 2 years older, their young cohort is highly comparable with our EA twins. Heritabilities of rest and stress levels of SBP, DBP, and HR were very similar between these two young cohorts, as were the newly emerging genetic factors and CVR heritability estimates for SBP and HR. In contrast to de Geus et al. (4), we observed newly emerging genetic effects for DBP during stress, whereas we were unable to confirm a significant amplification during stress of the genes that also acted on the resting levels for BP and HR. Taken together, our findings indicate that there will be some genes that show an effect on BP and underlying hemodynamics at rest and during stress, whereas others are expressed only when these traits are measured in stressful conditions.

Variation in genes within a number of pathways may moderate CV reactions to mental stressors, with genes encoding components of the parasympathetic and sympathetic nervous system, the renin-angiotensin-aldosteron system, and endothelial function as likely candidates (9,29,30). For example, Wang et al. (31) showed that a variant in the endothelin receptor type A gene led to higher SBP levels at rest and during acute mental stress. This gene, therefore, would be part of factor A in Figure 1. On the other hand, two variants in the endothelin-1 gene did not influence resting SBP but led to greater SBP increases to stress. This variation in the gene seems to be expressed only during stress and would be part of factor A*s* in Figure 1. Other studies that reported associations with stress-elicited BP levels focused on candidate genes from the sympathetic nervous system (32) and the renin-angiotensin-aldosteron system (33), whereas association studies with CVR were reviewed in Wu et al. (26).

Our Georgia Cardiovascular Twin study is one of the very few studies that investigated the genetic and environmental contribution to underlying hemodynamic regulators of BP (SV, CO, and TPR) for which we previously reported resting heritabilities (10) and emergence of novel genetic influences during adolescence (34). Heritabilities of these hemodynamic parameters were substantial for both rest and stress (between 31% and 58%), remained stable between conditions, and were comparable between ethnic groups. Stress-specific genetic effects were either small or absent in both EAs and AAs, whereas the effect of the common genetic factor influencing both resting and stress levels dampened for all hemodynamic variables and reached significance for SV in both EAs and AAs and cardiac and TPR index in AAs only. CVR heritability varied from 21% to 35%, except for SV and cardiac index in EAs, which showed no significant heritability.

Taken together, the present study has many strengths. First, we performed the analyses in both EAs and AAs to explore potential ethnic differences, which were seldom investigated by previous studies. Second, cardiovascular responses that were aggregated across the two mental stress tasks were used instead of the responses to each of the stressors separately. The former is more reliable because it reduces the relative influence of unique situational variance (18,35). Finally, the genetic and environmental influences of SV, CO, and TPR under stress were explored, providing more insight into the underlying hemodynamic regulators of BP.

Some limitations of the present study also need to be mentioned. First, because the Georgia Cardiovascular Twin Study comprises youth and young adults, the generalizability of these results to other adult populations remains to be determined. Second, our overall sample size was substantial for BP and underlying hemodynamics heritability estimates, but might not have had enough power to detect small ethnic or sex differences in heritabilities of the studied traits. Further twin studies with large sample sizes involving multiethnic groups would be needed to detect those.

In conclusion, we have shown that levels of BP and underlying hemodynamic variables at rest and during stress show substantial heritabilities comparable between ethnic groups. Our findings are in line with those from de Geus et al. (4) in suggesting that there will be some genes that show an effect on BP, HR, and underlying hemodynamic regulators at rest and during stress, whereas others are expressed only when these traits are measured in stressful conditions, suggesting that exposure to stress uncovers new genetic variance. In this regard, we expect that performing gene-by-stress interaction analyses in future gene-finding studies will be a promising way forward for detecting genes underlying BP regulation.

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CHAPTER 7

Genetic and environmental influences on blood pressure and body mass index in Han Chinese: a twin study

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ORIGINAL ARTICLE

Genetic and environmental influences on blood pressure and body mass index in Han Chinese: a twin study

Ting Wu[1,2], Harold Snieder[2,3], Liming Li[1], Weihua Cao[1], Siyan Zhan[1], Jun Lv[1], Wenjing Gao[1], Xiaoling Wang[3], Xiuhua Ding[3] and Yonghua Hu[1]

The familial aggregation of blood pressure (BP) may be partly due to the familial aggregation of obesity, caused by genetic and/or environmental factors that influence both. Gene–obesity interactions are expected to result in different heritability estimates for BP at different obesity levels. However, the latter hypothesis has never been tested. The present study included 1243 monozygotic and 833 dizygotic Han Chinese twins (mean ± s.d. age: 37.81 ± 9.82; range: 19.1–81.4) from the Chinese National Twin Registry. Body mass index (BMI) was used as the index of general obesity. The outcome measures were systolic BP (SBP) and diastolic BP (DBP). Quantitative genetic modeling was performed using Mx software. The SBP and DBP heritabilities were 46 and 30%, respectively. The positive correlations of BMI with SBP (r=0.26) and with DBP (r=0.27) were largely due to genetic factors (approximately 85%). Genetic factors, which also influence BMI, account for 6 and 7% of the total variance for SBP and DBP, respectively. The gene–obesity interaction analysis showed that both common and unique environmental influences on SBP increased with increasing levels of BMI, resulting in a lower heritability at higher BMI levels, whereas for DBP the heritability remained unchanged at higher BMI levels. Our results suggest that higher BMIs may reduce SBP heritability through a larger impact of environmental effects. These conclusions may be valuable for gene-finding studies.
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Keywords: blood pressure; heritability; interaction; obesity; twin

INTRODUCTION

Hypertension affects a large proportion of the adult population,[1] and is caused by complex interactions of environmental and genetic factors that vary across populations. Obesity is a well-established risk factor for hypertension.[2–4]

Twin studies have shown that underlying continuous traits for both hypertension and obesity are significantly heritable. Heritability estimates for both systolic blood pressure (SBP) and diastolic blood pressure (DBP) are between 40 and 60%,[5] and the heritability for body mass index (BMI) is also substantial.[6–8] These estimates raise the possibility that common genetic susceptibility may account for the association. Several previous twin and family studies have shown that the association between blood pressure (BP) and BMI is partly attributed to a common set of genetic factors,[5,9–11] although another study investigating the differences in monozygotic (MZ) twins showed that even in the absence of genetic influences, obesity may still be significantly associated with BP.[12] Moreover, most of these studies were performed in Caucasians and with relatively small sample sizes; studies conducted with Asian twins are rare.[13] Another possibility of interest is that obesity modifies the genetic susceptibility to hypertension. However, no study has hitherto quantified, such an effect of BMI, on the relative contribution of genetic and environmental factors on BP.

Twin studies provide a unique opportunity to provide evidence regarding genetic and environmental influences not only on the variation of individual traits but also on cross-trait correlations. Furthermore, study designs involving twins can examine evidence for gene–environment interactions.[14] Such information will help direct molecular, clinical and epidemiological studies on specific underlying causes of the disease. The present twin study is among the very few studies that investigate the shared genetic and environmental influences on BP and obesity, as well as the effect of gene–obesity interactions on these influences. Moreover, our study is the first to report such analyses in the Han Chinese population and uses a

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comparatively large sample size. The aims of this study were to (1) examine the relative influence of genetic and environmental factors on SBP, DBP and BMI; (2) investigate the extent to which the phenotypic correlations between BP and BMI may be explained by genetic or environmental factors using bivariate variance component models; and (3) investigate the extent to which BMI may modify the genetic influence on SBP and DBP in a large sample of adult twins from the Chinese National Twin Registry.

METHODS

Subjects

The present study comprised subjects from the Chinese National Twin Registry, which was established in 2001.[15,16] In total, 2111 subjects were recruited from Qingdao and Lishui, two cities located in the north and south of China, respectively. In this study, we excluded three triplets (nine individuals), 20 individuals who had type 2 diabetes, two pairs of twins who did not have sex information and one pair of twins who did not have BP measures. After the exclusions, 1243 MZ (620 pairs and three singletons) and 833 dizygotic (DZ) (414 pairs and five singletons) twins, including pairs of the same and the opposite sex (age, mean ± s.d.: 37.81 ± 9.82; range: 19.1–81.4), were included. Gender and ABO blood type were used for an initial screen of zygosity, and those twin pairs showing differences were categorized as DZ. Then ⩾4 Short Tandem Repeat markers were used to determine zygosity in the remaining twins.[16,17] Written informed consent was obtained from all participants before they entered the study, which was approved by the Ethics Committee for Human Subject Studies of the Peking University Health Science Center.

Measurements

BP was measured in seated subjects with a mercury-gravity sphygmomanometer according to standard protocols.[18] Each individual of a pair of twins was measured on the same day in the morning and was seated quietly for at least 5 min before measurement. Three measurements were taken for each subject with an interval of at least 30 s between measurements, and the mean value of the three measurements was used in the analysis. Subjects were asked to refrain from alcohol, coffee and vigorous exercise during the 24 h before the measurements, and they were also asked to fast for 12 h before the measurements. Height was measured to the nearest cm using a wall-mounted stadiometer. Weight (light clothing only) was measured to the nearest 0.1 kg using digital scales. BMI was calculated as weight divided by the square of the height ($kg\,m^{-2}$).

Statistical analysis

Structural equation modeling was the primary method of analysis. Structural equation modeling is based on the comparison of the variance–covariance matrices in MZ and DZ twin pairs and allows the separation of the observed phenotypic variance into its genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components. Dividing each of these components by the total variance yields the different standardized components of the variance. For example, the heritability (h^2) can be defined as the proportion of the total variance attributable to additive genetic variation.[19] We focused on the additive genetic effects and common and unique environmental effects because there was little evidence that correlations among MZ twins substantially exceeded twice those among DZ twins, which would indicate dominance variance.[19]

Sex differences were examined by comparing a full model, in which parameter estimates were allowed to differ in magnitude between men and women, with a reduced model, in which parameter estimates were constrained to be equal across the sexes. In addition to those models, a scalar model was tested. In the scalar model, the heritabilities were constrained to be equal across sexes, but total variances could be different.[20]

For the second purpose of the study, a bivariate path model, which is shown in Figure 1, was used. With this model, which made use of the 'Cholesky decomposition,' we can not only estimate the heritability of BMI ($(a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$) and BP ($((a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$) but also test whether the magnitude of the genetic influence differs for BMI and BP. We can further test whether the genes influencing BP are the same (that is, $a_{22}=0$?),

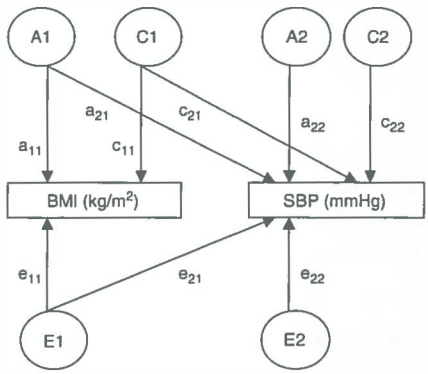

Figure 1 Path diagram for a bivariate model. For clarity only one twin is depicted. A1, A2=genetic variance components; C1, C2=common environmental variance components; E1, E2=unique environmental variance components; a_{11} through a_{22}=genetic path coefficients (or factor loadings) of which a_{22} represents specific genetic influences on SBP; c_{11} through c_{22}=common environmental path coefficients (or factor loadings) of which c_{22} represents specific common environmental influences on SBP; e_{11} through e_{22}=unique environmental path coefficients (or factor loadings), of which e_{22} represents specific unique environmental influences on SBP. Formulas for the different heritability estimates are as follows:

h^2 total (BMI) $= a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$

h^2 total (SBP) $= (a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

h^2 shared (SBP explained by BMI) $= a_{21}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

h^2 specific (SBP) $= a_{22}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$

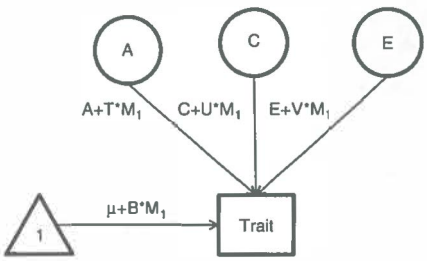

Figure 2 Partial path diagram for the basic gene-environment interaction model. A=additive genetic effects; C=common environmental effects; E=unique environmental effects; M=moderator (BMI in the present study); T=moderated component of A; U=moderated component of C; V=moderated component of E; B=linear effects of moderator on mean (forced entry).

partly the same (that is, $a_{21}\neq 0$ and $a_{22}\neq 0$?) or entirely different (that is, $a_{21}=0$?) from those for BMI. If they are partly the same, this bivariate model allows further determination of the amount of overlap between the genes that influence BP and BMI by calculating the genetic correlation between the two traits ($r_g=COV_A$ (trait 1, trait 2)$/\sqrt{(V_A\text{trait1}*V_A\text{trait2})}$). The shared and unique environmental correlations can be calculated in a similar manner.[19,21]

We then fit the gene-environment interaction models as described by Purcell[14] using BMI as a continuous moderator and incorporating all available twin pairs (Figure 2). In this gene-environment interaction model, the phenotypic variance of the outcome variables (SBP and DBP) was portioned into A, C and E components, with the path coefficients associated with each variable expressed as linear functions of the moderator (for example, A+T×M1, C+U×M1 and E+V×M1), in which M1 represents the value of the moderator

and B represents the linear effects on the outcome. Because we were primarily interested in the effects of BMI on the variance components, we forced B into the model to guard against model misspecification. That is, by forcing the effect of BMI into the model, we guarded against detecting the GxE that is actually due to gene-environment correlation (r_{GE}). A significant compromise of model fit when parameters T, U and V were fixed to zero reflected evidence of significant moderation of additive genetic, common environmental and unique environmental variance by BMI, respectively. For example, a significant moderation of additive genetic variance alone would suggest that the magnitude of the heritability of SBP changes as the moderator increases or decreases. Variance components were only tested for significance if the respective interaction terms had been dropped from the model; for example, A was not tested unless T was not significant, to avoid modeling interactions in the absence of the main effects. In the final model, each parameter contributed significantly to the model fit ($P<0.05$). BMI may be correlated with the genetic effects on BP (r_{GE}) rather than modifying the genetic effects on BP (G× E). However, entering BMI in the means model to allow for a main effect would effectively remove from the covariance model any genetic effects that may be shared between BP and BMI (a_{21} in Figure 1). Thus, any interactions detected will not be due to gene–environment correlation (r_{GE}), but will instead be interactions between BMI and the variance components specific to BP. We further performed stratified analyses in twin pairs concordant for normal weight or overweight to confirm the results from the above analysis. Overweight in our population of Han Chinese was defined as BMI≥24 kgm^{-2}.[2,22,23]

Before the analysis, 15 and 10 mm Hg were added to SBP and DBP, respectively, for individuals using antihypertensive medication because this addition was shown to reduce bias and improve statistical power.[24,25] However, performing all analyses after the exclusion of 39 subjects who were currently taking antihypertensive medication yielded virtually identical results. SBP, DBP and BMI were log-transformed to obtain a better approximation of the normal distribution. The effects of age, gender and study site were regressed for the log-transformed SBP and DBP before using the residuals in model fitting. To obtain the heritabilities for SBP and DBP independent of obesity, we adjusted for BMI in the univariate analysis. For the gene-BMI interaction analysis, only SBP and DBP were log-transformed. The significance of moderator effects T, U and V and variance components A and C were assessed by testing the deterioration in model fit after each term was dropped from the full model. Standard hierarchic χ^2-tests were used to select the best-fitting models in combination with Akaike's Information Criterion (χ^2-2d.f.). The model with the lowest Akaike's Information Criterion reflects the best balance of goodness of fit and parsimony.[19] Preliminary analyses were done using STATA 10.0 (Stata Corp., College Station, TX, USA). Genetic modeling was conducted with Mx, a computer program specifically designed for the analysis of twin and family data.[26]

RESULTS

Sample and demographics

The general characteristics of the male and female twins are presented in Table 1. Men had higher SBPs and DBPs than women. More women lived in the city of Qingdao compared with men. Subjects who lived in Qingdao had higher SBPs and DBPs ($Ps<0.001$) than those living in the city of Lishui. None of the traits showed significant differences between MZ and DZ twins.

Table 2 presents the twin correlations for all traits of each sex-by-zygosity group. For SBP and DBP, the results are shown before and after adjustment for BMI. The MZ correlations were consistently higher than the DZ correlations, indicating an important contribution of genetic factors. The DZ correlations for all traits were more than half of the corresponding MZ correlations, suggesting an absence of dominance (D) effects. There was no substantial change in the correlations after adjustment for BMI.

Univariate model

Parameter estimates of the best-fitting models for SBP, DBP and BMI are shown in Table 3. All three traits were significantly heritable, with heritabilities of 46% for SBP, 30% for DBP and 74% for BMI. For SBP and DBP, the model, including additive genetic, common and unique environmental (ACE) effects without sex differences, provided the best fit. Heritability of SBP and DBP decreased after adjustment for BMI, indicating that BP shared some genetic factors with BMI to some extent. For BMI, we also tested for the presence of a common environmental effect, but dropping this effect had virtually no effect on the model fit. A scalar sex effect was observed for BMI, with women showing larger total variability than men, but there was equal heritability across the sexes.

Table 1 General characteristics and blood-pressure related variables of study subjects by gender

	Male	*Female*	*Sex difference, P*
Subjects, *n*[a]	1092	984	
Age, years	38.73±10.6	36.79±8.71	NS
Study site (Qingdao %)	47.5%	58.5%	<0.001
Zygosity (MZ %)	61.1%	58.7%	NS
BMI, kgm^{-2}	22.9±3.02	23.0±3.39	NS
SBP, mm Hg	123.0±16.9	114.8±15.7	<0.001
DBP, mm Hg	82.6±11.1	77.3±10.4	<0.001
Antihypertensive medication use[b]	22 (1.99%)	17 (1.71%)	NS

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DZ, dizygotic; MZ, monozygotic; SBP, systolic blood pressure.
Data are mean±s.d. unless stated otherwise.
[a]Eight singletons, 620 MZ and 414 DZ pairs, two missing values for BMI in male and one in female, one missing value (outlier) for SBP in male.
[b]Antihypertensive medication use in the most recent 2 weeks.

Table 2 Twin correlations of each sex-by-zygosity group for SBP, DBP and BMI

Measure	*MZM*	*DZM*	*MZF*	*DZF*	*DOS*
N pairs	332	111	288	103	200
SBP, mm Hg[a]	0.70/0.70	0.46/0.43	0.65/0.63	0.47/0.49	0.42/0.43
DBP, mm Hg[a]	0.66/0.64	0.50/0.46	0.60/0.58	0.42/0.43	0.40/0.39
BMI, kgm^{-2}	0.73	0.50	0.76	0.42	0.29

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DOS, dizygotic opposite sex; DZM, dizygotic male; DZF, dizygotic female; MZF, monozygotic female; MZM, monozygotic male; SBP, systolic blood pressure.
[a]Twin correlations for SBP and DBP are shown before and after adjustment for BMI. All measures were log-transformed and adjusted for age, sex and study site.

Bivariate model

We performed a bivariate model fitting to estimate to what extent the phenotypic correlations can be explained by genetic or environmental factors that influence both BP and obesity (Figure 1). BMI was significantly correlated with both SBP (0.26) and DBP (0.27), reflecting higher levels of BP at higher levels of BMI. Genetic correlations for BP and BMI were substantial (0.38 and 0.48 for SBP and DBP, respectively), whereas unique environmental correlations were somewhat weaker, but still highly significant (0.17 and 0.12 for SBP and DBP, respectively) (Table 4). The decomposition of the phenotypic correlations into their genetic and environmental parts showed that they were largely (82% for SBP and BMI, 86% for DBP and BMI) due to genetic factors.

Figure 3 presents sources of variance of BP based on the best-fitting bivariate models. Around 38% percent of the total variance for SBP and 24% for DBP could be attributed to specific genetic factors that

Table 3 Parameter estimates and 95% CIs of best-fitting models for SBP, DBP and BMI

Measures		*h^2 (95%CI)*	*c^2 (95%CI)*	*e^2 (95%CI)*	*Sex effects*
SBP, mm Hg	Model 1	0.46 (0.30–0.62)	0.22 (0.06–0.36)	0.32 (0.29–0.37)	
	Model 2	0.41 (0.26–0.58)	0.25 (0.09–0.39)	0.34 (0.30–0.38)	
DBP, mm Hg	Model 1	0.30 (0.14–0.48)	0.31 (0.15–0.46)	0.38 (0.34–0.43)	
	Model 2	0.27 (0.10–0.45)	0.32 (0.16–0.47)	0.41 (0.37–0.46)	
BMI, kgm^{-2}		0.74 (0.71–0.77)		0.26 (0.23–0.29)	k^2=0.81

Abbreviations: BMI, body mass index; c^2, common environmental effects; CI, confidence interval; DBP, diastolic blood pressure; e^2, unique environmental effects; h^2, heritability; k^2, scalar sex effect; SBP, systolic blood pressure.
All (non-standardized) variance components for males are constrained to be equal to a scalar multiple, k^2, of the female variance components, such that $h_m^2=k^2h^2$, $c_m^2=k^2c_f^2$ and $e_m^2=k^2e_f^2$. As a result, the standardized variance components, such as heritabilities, are equal across sexes.
BMI was log-transformed and adjusted for age, sex and study site.
Model 1: SBP and DBP were log-transformed and adjusted for age, sex and study site.
Model 2: Model 1+ adjustment for BMI.

Table 4 Phenotypic (r_P), genetic (r_A) and unique environmental (r_E) correlations from the best-fitting bivariate models

	Phenotypic correlation		*Additive genetic correlation*		*Unique environmental correlation*		*Proportions of r_P*
	r_P	*95%CI*	*r_A*	*95%CI*	*r_E*	*95%CI*	*A/E*
SBP and BMI	0.26	0.22–0.31	0.38	0.28–0.49	0.17	0.10–0.24	0.82/0.18
DBP and BMI	0.27	0.22–0.31	0.48	0.35–0.68	0.12	0.05–0.19	0.86/0.14

Abbreviations: A, additive genetic factor; CI, confidence interval; E, unique environmental factor; h^2, heritability.
All phenotypes were log-transformed and adjusted for age, sex and study site.

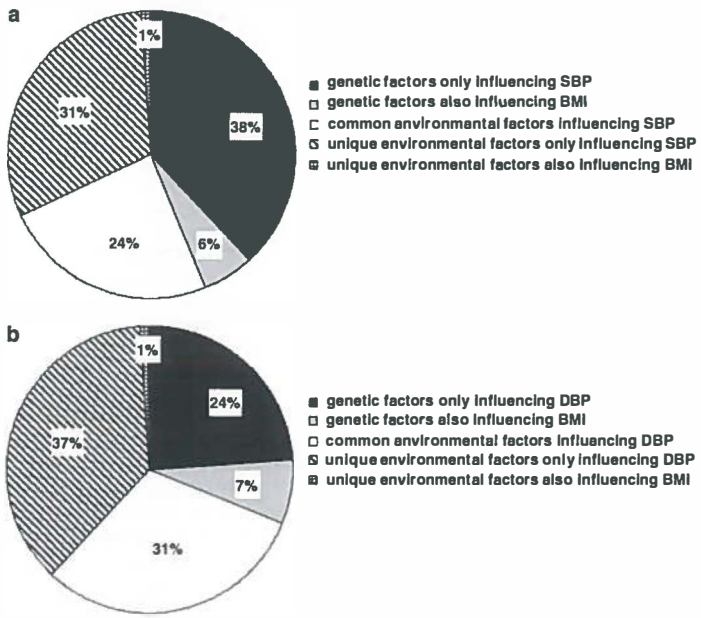

Figure 3 A decomposition of the variance of BP in its genetic and environmental components (that is, genetic and environmental sources of individual differences in BP) is shown for SBP (a) and DBP (b). We could further discriminate between genetic and environmental factors that also influenced BMI or were specific to BP.

only influenced BP. Genetic factors that also influenced BMI contributed 6% to the total variance for SBP and 7% for DBP. Comparatively, environmental factors that also influenced BMI contributed very little to the total variance for SBP and DBP (1%).

Gene–environmental interaction model

Comparative model fittings that tested the extent to which BMI served as a moderator of SBP and DBP are presented in Table 5. Gene–BMI analyses were collapsed across genders because heritability estimates of

Table 5 Comparative model fitting for BMI as a continuous moderator of SBP and DBP

	Model fitting			Comparative model fitting			
	Model	-2LL	d.f.	Δ-2LL	Δd.f.	P-value	AIC
SBP							
1.Full	ACETUVB	6067.531	2054				
2.T=U=V=0	ACE8	6092.519	2057	24.988	3	<0.001	18.988
3.V=0	ACETU8	6074.529	2055	6.999	1	0.008	4.999
4.U=0	ACETV8	6068.573	2055	1.042	1	0.307	-0.958
5.T=0	**ACEUVB**	**6067.540**	**2055**	**0.010**	**1**	**0.922**	**-1.990**
6.U=V=0	ACET8	6074.561	2056	7.031	2	0.030	3.031
7.T=V=0	ACEU8	6077.025	2056	9.494	2	0.009	5.494
8.T=U=0	ACEV8	6074.966	2056	7.436	2	0.024	3.436
DBP							
1.Full	ACETUVB	6355.688	2054				
2.T=U=V=0	**ACEB**	**6363.211**	**2057**	**7.524**	**3**	**0.057**	**1.524**

Abbreviations: -2LL, -2-log likelihood; A, additive genetic variance; AIC, Akaike's Information Criterion; B, linear effects of BMI on means of the outcome variables; BMI, body mass index; C, common environmental variance; DBP, diastolic blood pressure; d.f., degrees of freedom; E, unique environmental variance; SBP, systolic blood pressure; T, moderation of additive genetic variance by BMI; U, moderation of common environmental variance by BMI; V, moderation of unique environmental variance by BMI.
All models were compared with the full model (model 1).
SBP and DBP were log-transformed and adjusted for age, sex and study site.
Best fitting models are in bold.

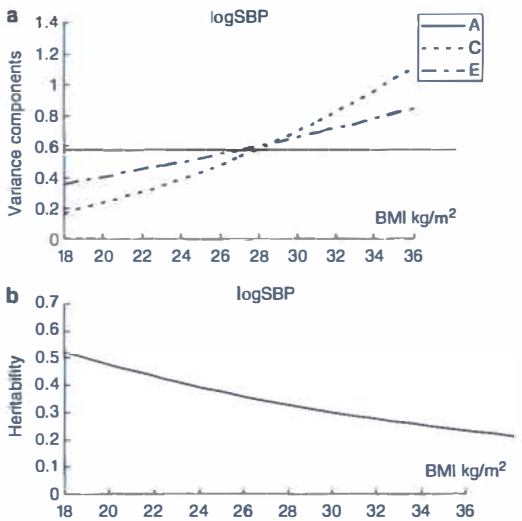

Figure 4 (a) Change of additive genetic, common environmental and unique environmental variances of SBP with increasing level of BMI. (b) Change of heritability of SBP with increasing level of BMI.

SBP and DBP were not significantly different between men and women (Table 3). The best-fitting model for SBP was the ACEUVB model in which the moderators of the common (U) and unique environment (V) components contributed significantly. As shown in Figure 4a, both the common and the unique environmental influences on SBP increased with BMI, which resulted in a lower heritability estimate for SBP at higher BMI levels; for example, the heritability for SBP is 0.47 at BMI=20 kg m^{-2} and 0.32 at BMI=30 kg m^{-2} (Figure 4b). For DBP, the best-fitting model was the ACEB model in which none of the moderators contributed significantly.

Table 6 Twin pair correlations and univariate twin structural equation model parameter estimates (unstandardized) for BP, normal weight and overweight subjects

	N (pairs)	Correlations					Total
Variables	MZ/DZ	r_{MZ}	r_{DZ}	a^2	c^2	e^2	variance
SBP							
Normal weight	357/195	0.68	0.43	0.53	0.27	0.40	1.20
Overweight	140/76	0.65	0.52	0.53	0.60	0.58	1.71
P-value[a]				0.989	0.293	0.006	
DBP							
Normal weight	357/195	0.59	0.42	0.41	0.46	0.63	1.50
Overweight	140/76	0.63	0.50	0.33	0.62	0.59	1.54
P-value[a]				0.796	0.588	0.526	

Abbreviations: a^2, additive genetic effects; BP, blood pressure; c^2, common environmental effects; DBP, diastolic blood pressure; DZ, dizygotic; e^2, unique environmental effects; MZ, monozygotic.
[a]*P*-value: *P* value is shown for the χ^2 difference (1 d.f.) between the full ACE model with BMI differences and the ACE sub-model, in which normal weight and overweight parameter estimates are set equal, that is, a^2 (normal weight)=a^2 (overweight), then c^2 (normal weight)=c^2 (overweight) and then e^2 (normal weight)=e^2 (overweight), respectively.
Normal weight: BMI <24 kg m^{-2}; overweight: BMI ≥24 kg m^{-2}.
SBP and DBP were log-transformed and adjusted for age, sex and study site.

The change in variance components of SBP with different BMI levels was confirmed by stratified analysis in twin pairs concordant for normal weight and for overweight, as shown in Table 6. As a result, the heritability was 14 percentage points lower for SBP among twins concordant for overweight (h^2=0.31) relative to twins concordant for normal weight (h^2=0.45). Moreover, the unchanged heritability of DBP in the interaction model was confirmed by stratified analysis, which showed the same heritability (h^2=0.26) in subjects with normal weight and overweight.

DISCUSSION

The aims of this study included estimating the relative influence of genetic and environmental factors on BP and BMI. Furthermore, we investigated the genetic and environmental overlap between BP and BMI and the extent to which BMI may modify the effect of genetic factors on SBP and DBP in a large sample of adult twins from the Han Chinese population. The current study suggests that genetic and environmental influences on SBP may vary as a function of BMI.

The present study, for the first time, reported the heritability of BP in a large sample of adult Han Chinese twins and further investigated sex effects. The major difference between this study and former twin studies is that we found a significant contribution of the common environment to BP. The environmental factors common to twins for SBP and DBP explained 22 and 31%, respectively, of the total variance. Because familial resemblance could be explained by sharing the environment in addition to sharing genes, the heritability estimates for BP in the present study were somewhat lower than those reported in the previous twin studies.[5,27] Part of the explanation might be the diversity of genetic backgrounds and/or differences in the environmental effects between populations. The present study was performed in Han Chinese, whereas previous studies were mostly performed in white twins.[5] In addition, the common environmental influences detected in this study may be attributable to different sample sizes. Hopper[28] convincingly argued that most twin studies simply lacked the power to detect moderate-size influences of the common environment. A few studies that either had large sample sizes or used a

more powerful multivariate approach did find a small contribution by the common environment of approximately 10–20%.[5] For example, a large family study, including two-generation families, reported that common environmental effects accounted for 31 and 23% of the variance in SBP and DBP, respectively.[11] The present study is one of the largest twin studies to date that investigates the relative contribution of genes and environment on BP. Thus, our results confirm that large sample sizes are needed to detect moderate influences of the common environment in twin studies. However, we did not observe any sex differences for BP. Our results were in line with most previous twin studies in which heritability estimates for men and women are remarkably similar.[5] We also detected a substantial heritability of 0.74 for BMI, which is consistent with previous findings.[6,29,30] The heritability estimates of BP decreased after adjustment for BMI, suggesting that the heritability of BP is partly dependent on BMI.

We further confirmed the above conclusion by performing bivariate variance components analyses. Our results showed that genetic factors accounted for a large portion of the correlations between BP and BMI (approximately 85%), indicating that these phenotypes, as expected, had a set of genes in common.[31] The percentage of total BP variance caused by genetic effects common to BP and BMI was 6 and 7% for SBP and DBP, respectively. Schieken *et al.*[32] addressed the same issue in a pediatric population of 11-year-old twins. They observed a significant correlation between SBP and BMI (0.29) that could largely be explained by common genes rather than common environmental effects influencing both traits. The percentage of total SBP variance caused by genetic effects common to SBP and BMI was 8%, which is consistent with our results. McCaffery *et al.*[9] also reported that genetic and, to a lesser extent, non-shared environmental factors contribute to the covariation of SBP and BMI in young adult twins. Another study performed in African-American twins found sex differences, showing that 3.1% of the total variance in SBP was in common with BMI in males and 6% in females, while for DBP, 6.1% was in common with BMI in males and 3.7% in females.[10] The consistent results of these studies across different ethnicities and age groups confirm that part of the genetic variation in BP can be explained by genes for obesity.

A novel finding of this study is that the genetic and environmental influences on SBP vary as a function of general obesity. The influence of common and unique environmental factors increases with increasing levels of BMI, resulting in a decreased level of heritability. This result seems to suggest that higher BMI levels may reduce the penetrance of genetic vulnerability to SBP through a larger impact of environmental effects. Using models similar to the present study, McCaffery *et al.*[33] reported a higher heritability of hypertension with more years of education. Typically, socioeconomic status, as indexed by education attainment, occupation and income, is inversely associated with BMI levels, even in developing countries.[34,35] In other words, our results are consistent with those from McCaffery's study, showing reduced heritability in the high-risk environment; that is, heritability was reduced with higher BMIs in our study and lower education levels in McCaffery's. Although the specific mechanism by which BMI affects the heritability of BP cannot be determined from this study, it is well known that several environmental and behavioral factors that predict BP levels, such as unhealthy diets and lack of physical activity, are more prevalent among groups with higher BMIs. Within the twin design, this type of effect may manifest itself as an enhancement of the environmental effect relative to the genetic effect, resulting in reduced heritability.

Our results have some implications for further efforts to find genes underlying BP levels. Although BP shows a substantial heritability of approximately 40–60%,[5] the findings for candidate genes have been difficult to consistently replicate. Two recent large-scale genome-wide association studies[36,37] identified common variants in 13 loci associated with BP that each explained only 0.05–0.10% of the variance,[36] and only about 1% was associated with BP when aggregated over all the loci.[37] Thus, the vast majority of the genetic contributions to variation in BP remain unexplained. One explanation for the difficulty in finding genes for BP is that the expression of genes may vary as a function of environmental exposures. This would mean that their effects could only be found in the presence of certain environments. Our results provided some evidence for this because genetic variation of SBP was higher in subjects with normal BMI. This suggests that in the Han Chinese, the selection of subjects with normal BMIs would increase the power required to find genes for SBP. Future genome-wide association studies may want to rigorously measure environmental exposures and perform gene–environment interaction analyses to enhance the chances of finding genes.[38]

There are many strengths of this paper. First, our study was based on a large sample of adult male and female twins of Han Chinese ethnicity and is one of the largest twin studies for BP.[5,27] Second, BMI was measured in an objective way rather than self-reported. In our study, BP was measured three times according to standard procedures, and we used the mean values in the analyses to decrease measurement error. There are also some potential limitations. First, the Chinese Twin National Registry is predominantly of Han ethnicity. Thus, the generalizability of these results to other ethnicities remains to be determined. Second, although we treated BMI as an environmental moderator in the statistical models, BMI is not a purely environmental factor. We have shown in our study that BMI is both substantially heritable and shows genetic and environmental overlap with BP. At the same time, however, the interaction model suggests that BMI influenced SBP heritability through a larger impact of environmental effects. By entering BMI into the means model to allow for a main effects model, we effectively removed from the covariance model any genetic effects that are shared between SBP and BMI (a_{21} in Figure 1). Any interactions detected were not due to gene–obesity correlation, which is essential when interpreting and generalizing our results. Third, the model used to test for interactions, including a continuously measured environmental variable, is relatively new. However, splitting the sample into a normal and an overweight group confirmed our findings. Fourth, we did not include other risk factors for hypertension (for example, smoking, alcohol consumption and salt intake), which may have some influence on the modifying effects of BMI on BP.

In conclusion, our findings show that genes for obesity explain part of the genetic variation in BP. These results will provide important information for strategies for the discovery of specific genes or environmental factors that impact BP, BMI or both, especially in Asian populations. Our results further suggest that BMI levels are associated with the heritability of SBP. In this regard, we expect that performing gene–obesity interaction analyses in future genome-wide association studies will enhance the efficiency of detecting BP susceptibility genes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CHAPTER 8

General discussion

The main aim of the research described in this thesis was to apply different gene-environment (GxE) interaction methods as mentioned in the general introduction on risk factors for asthma and cardiovascular disease. The thesis started in **Chapter 2** with a prospective candidate gene study, aiming to give more insight in interaction between maternal passive smoking and maternal metabolic genes on infant birth weight. Low birth weight is a known risk factor for childhood asthma and cardiovascular diseases in adulthood such as hypertension and coronary artery disease. The remaining five chapters of the thesis consisted of a number of twin studies with the purpose to extend applications of twin modeling to explore effects of GxE interactions on intermediate phenotypes for asthma and cardiovascular disease.

Below we first discuss GxE interactions on infant birth weight and intermediate asthma phenotypes before continuing with GxStress interaction on epinephrine and norepinephrine excretion, environmental challenges on BP and hemodynamic measures and modifying effects of obesity on heritability of BP. Next, we provide some methodological considerations for exploring GxE interaction in candidate gene association studies compared with twin studies. Finally, we discuss implications from the studies conducted and provide future perspectives. **Table 1** gives an overview of the key findings of each of the twin studies described in **Chapters 3** to **7**.

GxE Interaction on Infant Birth Weight

Low infant birth weight is an independent risk factor for childhood asthma (1, 2) and subsequent cardiovascular diseases (3). The association between low birth weight and increased BP, although modest, has been well established as shown by a meta-analysis of 34 studies: BP is lower by 1-2 mmHg for every kg increase in birth weight for children and the effect size increases to about 5 mmHg/kg in elderly people (4). The fetal programming hypothesis states that this association is due to intrauterine malnutrition (reflected by low birth weight), which increases the risk of a number of chronic diseases in later life including hypertension. Current studies suggest that both environmental and genetic factors in various periods of life may underlie the complex associations of fetal growth retardation and low birth weight with cardiovascular disease in later life (3).

Maternal smoking during pregnancy is strongly associated with increased risks of preterm birth or a small size for gestational age at birth (5). It has been estimated that, in Western countries, 30 percent of children with low birth weight can be explained by

exposure to tobacco smoke during pregnancy (6, 7). The effects of maternal smoking on fetal growth differ between individuals. These differences in effects might be explained by maternal genetic predisposition. Results of the study presented in **Chapter 2** (8) showed that variants of metabolic enzyme genes cytochrome P-450 1A1 (*CYP1A1*) and epoxide hydrolase 1 (*EPHX1*) modified the association between maternal passive smoking and infant birth weight in the Chinese population. In the passive smoking group, there was a remarkably lower birth weight for the *CYP1A1* MspI C/C6235 compared to the T/T6235 genotype (156.3 g) and for both the *EPHX1* Tyr/His113 (93.8 g) and the His/His113 (244.6 g) compared to the Tyr/Tyr genotype. The *CYP1A1* MspI and *EPHX1* genotypes modified the association between maternal passive smoking and infant birth weight in this study, indicating GxE interaction. Our results are consistent with another study conducted in the Korean population that showed maternal exposure to environmental tobacco smoke negatively affects neonatal birth weight modified by maternal metabolic genotypes for *GSTM1* and *GSTT1* genes (9). These observations bear biologic plausibility. There are two primary detoxification enzymatic pathways in the liver in which chemicals are eliminated or neutralized, known as phase I and phase II reactions. The phase I reaction involves oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug and can generate metabolites which are more chemically active and potentially toxic. Most of phase II reactions take place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. While mechanisms leading to intrauterine growth restriction after in utero tobacco exposure have generally been attributed to chronic fetal hypoxia, it is known that nicotine, cotinine, and DNA adducts cross or collect in the placenta of smokers. Phase I gene products, such as aryl hydrocarbon hydroxylase encoded by *CYP1A1*, is a well-studied phase I enzyme and is particularly relevant to the metabolism of chemicals in cigarette smoke. Detoxification of these epoxides may occur through conjugation with certain endogenous functional groups or by hydration, catalyzed by epoxide hydrolases in phase II. The end product becomes a stable hydrophilic compound that can easily be excreted (10). Our results support that the variation in maternal susceptibility to smoking-related growth restriction results from the diminished ability to convert and excrete these reactive intermediates.

Recently, a genome-wide association study (GWAS) meta-analysis identified a common genetic variant, rs1051730, located within the 15q25 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNB4), to be associated with smoking quantity (11).

Subsequently, a population-based prospective cohort study among 3,563 European mothers suggested that the same maternal SNP modifies the associations of maternal smoking during pregnancy resulting in impaired fetal length and weight (12).

Gene-maternal smoking interaction studies are of increasing importance in investigating fetal growth retardation and low birth weight. Although smoking cessation and avoidance of exposure to passive smoking should be advised to all pregnant women, a genetic risk profile might help us to target more effectively those at higher risk of growth retardation and adverse pregnancy outcomes. Future research is needed on exploring the maternal-infant gene-gene interactions. Furthermore, future studies aimed at illuminating the complex interplay of genomic–epigenomic–environmental interactions may help dissect multifactorial etiologies and identify at-risk populations for these common adverse outcomes of pregnancy.

GxE Interaction on Intermediate Asthma Phenotypes-Twin Study

Asthma is a complex multifactorial disorder involving a variety of different mechanisms. Understanding the interplay between different pathways underlying asthma is central to the discovery of targeted treatments and interventional measures. However, little has changed in asthma treatment over the past five decades. There is evidence for a strong genetic component of asthma, but genetic studies have produced heterogeneous results with little replication, with most of the heritability remaining unexplained. The rapid increase in asthma prevalence over the last three decades suggests that environmental exposures play an important role, but there is a considerable heterogeneity in the results describing the effect of different environmental exposures. One of the reasons for lack of replication in genetic association studies and those of environmental exposures could be the failure of consideration of GxE interactions, that is, the respons to environmental factors is modulated by the genetic susceptibility of individuals.(13). In addition, many studies rely on oversimplified phenotypes often derived through aggregation of several heterogeneous conditions (e.g., 'physician-diagnosed asthma'). Studies on objective intermediate phenotypes specific to asthma are pivotal and needed to reveal the pathways underlying the disease. However, the present knowledge of the relationship between intermediate asthma phenotypes is limited. Twin studies can provide important clues, since they can be used to quantify the genetic and environmental sources of variation in human traits. Furthermore, information from multivariate datasets enables us to examine the degree of genetic and environmental overlap

between different traits (14).

In **Chapter 3**, we showed that the provocation concentration producing a 20% fall in FEV_1 (PC_{20}), skin prick test (SPT) and specific Immunoglobulin E (IgE), which are intermediate asthma phenotypes reflecting direct responses to environmental stimuli such as methacholine and 11 common allergens (e.g., house dust mite, storage mite and mixed tree pollens et al.), have moderate heritabilities of 0.47, 0.56 and 0.60, respectively (15) (**Table 1**). These results confirmed evidence from other independent studies showing that innate immunity genes (particularly CD14, Toll-like receptor (TLR) 2 and TLR4, critical mediators of responses to bacteria in the extracellular space) play a prominent role in GxE interaction studies of asthma-related phenotypes (16). A large body of epidemiological literature supports the interaction between microbial burden and prevalence of allergies (17). A polymorphism in the *CD14* gene has provided one of the clearest examples of the complexity of GxE interactions so far, including the classical finding of opposite effects of a given genotype depending on the quantity and/or quality of the relevant microbial load (18, 19)

Additionally, we found that much of the phenotypic correlations between these objective asthma related traits are attributable to genetic factors (most were above 70%). At the same time remarkable environmental overlap (0.70) was seen between specific IgE and SPT in our study, perhaps because these traits represent responses to similar allergens. What deserves to be mentioned is that SPT and PC_{20} also showed a considerable degree of environmental overlap (0.44), which explained the main part of the phenotypic correlation between these two traits, perhaps also because both of them represent a direct response to environmental stimuli. This is plausible given the observation that inhalation of allergens to induces a more severe hyperresponsiveness in responsive individuals, or even renders a non-responsive atopic individual hyperresponsive during the allergic season (20, 21). These findings widen the insight of the origins of clinical homogeneity for asthma-related traits and provide some clues and directions for gene finding studies.

GxStress Interaction on Epinephrine / Norepinephrine

Chronic environmental stress, together with the effects of genetic predisposition and other mediating factors (e.g., lifestyle and personality), may exert its influence on preclinical markers of essential hypertension through the activation of hypothalamus pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) pathways (22). The

catecholamines epinephrine (E) and norepinephrine (NE) mediate the early stress response via the SNS and may play a key role in homeostatic blood pressure (BP) control (23). Data from the Georgia Cardiovascular Twin Study provided a unique opportunity to test the above hypothesis. In the study described in **Chapter 4**, we expected to observe an effect of urinary E and NE excretion as measures of chronic stress exposure on BP levels.

In **Chapter 4**, we used overnight urinary excretion rates of NE ($U_{NE}V$) and E (U_EV) as measures of basal sympathetic activity and observed significant heritabilities for $U_{NE}V$ (0.68) and U_EV (0.74) without ethnic and gender effects (24) (**Table 1**). Not surprisingly, we also observed a high genetic correlation of 0.86 between $U_{NE}V$ and U_EV. Similar results were reported recently showing that both plasma and urinary E and NE were significantly heritable and ranged from 0.49 to 0.72 (25, 26). Several studies, which reported associations between genetic polymorphisms and catecholamine secretion confirmed our results (26-28). These results suggest that genetic factors are strong determinants of basal sympathetic activity. Unlike our original expectation, there was no clear pattern of correlations for $U_{NE}V$ and U_EV with BP measures in European Americans (EAs). However, African Americans (AAs) showed some moderate inverse correlations, possibly because AAs excreting more catecholamines are also better capable of (down)regulating their BP, for example through more efficient sodium excretion and volume regulation.

Environmental Challenges on BP and Hemodynamic Measures

Individual differences in the cardiovascular response to stress play a central role in the reactivity hypothesis linking frequent exposure to psychosocial stress to adverse outcomes in cardiovascular health. In many studies, BP is measured under certain standardized environmental challenges. For example, BP can be measured under mental or physical stress. In the 1990s, Turner and Hewitt (29, 30) reviewed a number of early twin studies that explored the genetic and environmental origins of individual differences in heart rate (HR) and BP reactivity to psychological challenge. Their conclusion was that HR and BP reactivity are substantially heritable. Additional twin studies of cardiovascular reactivity have since confirmed heritability of HR and BP reactivity, but estimates for systolic BP (SBP), Diastolic BP (DBP) and HR reactivity have been very different across studies for the same task or, within the same study, across different tasks, and have ranged from 0.00 to 0.85 (31-37). In our study presented in **Chapter 5**, we used meta-analysis of twin resemblance in SBP, DBP and HR reactivity to

show that cardiovascular stress reactivity to mental stressors and the cold pressor test are heritable traits (38). For SBP reactivity to the mental stressors, the pooled heritability across all studies ranged from 0.26 (males) to 0.38 (females). SBP reactivity to the cold pressor test yielded comparable heritability estimates ranging from 0.21 (males) to 0.33 (females). For DBP reactivity, heritability to the cold pressor test was higher (0.55) than that to mental stress even after including dominance variation (broad heritability of 0.29). Heritability estimates for HR reactivity to mental stress (0.43) and the cold pressor test (0.45) were very similar (**Table 1**).

However, a limitation of most twin studies performed so far, and hence of the meta-analysis based on these studies, is that they analyzed reactivity as a change score. That way, the heritability estimates will reflect an inseparable mix of newly emerging genetic or environmental influences during stress and an amplification or dampening of genetic or environmental influences already present at rest.

Bivariate quantitative genetic twin models that include both challenged and unchallenged conditions can distinguish between genetic and environmental effects on levels of the challenged and unchallenged phenotypes. In fact, such a challenged phenotype may be more heritable than its unchallenged counterpart, potentially offering important advantages for gene finding studies. De Geus et al. used the bivariate approach mentioned above to investigate the cardiovascular response during a stress challenge and test for the existence of gene-by-stress interaction within the context of a classic twin study (35). Cardiovascular reactivity to stress, measured as the averaged response to a choice reaction time and mental arithmetic test, was assessed for SBP, DBP and HR in 160 adolescent and 212 middle-aged twin pairs. Genetic factors significantly contributed to individual differences in resting SBP, DBP and HR in the adolescent and middle-aged cohorts. The effect of these genetic factors was amplified by stress for SBP, DBP and HR in the adolescent cohort and for SBP in the middle-aged cohort. In addition, stress-specific genetic variation emerged for SBP in the adolescent cohort and for HR in both groups. It can be concluded that exposure to stress may uncover new genetic variance and amplify the effect of genes that already influence the resting level (35).

In **Chapter 6**, we replicated the work by De Geus et al. (35) in adolescents and extended it to another ethnic group by including not only EA but also a group of AA twins. Furthermore, we extended it to include cardiac output (CO) and total peripheral resistance (TPR) of the systemic vasculature as hemodynamic determinants of BP,

because a given increase in BP can be the result of an increase in CO, an increase in TPR, or a combination of alterations in both parameters. No study hitherto has addressed the heritability of changes in hemodynamic variables in response to stress. We observed that heritability indices for levels at rest and during stress were high (most were above 50%, **Table 1**) and comparable between ethnic groups. We observed increases in heritability for BP and HR from rest to stress that were mostly explained by newly emerging genetic influences on the added stress component. The stable component of the heritability was accounted for by a simultaneous decrease in genetic and environmental variances. Several candidate gene studies on the associations with stress-elicited BP levels also confirmed our results (26, 39, 40). We further observed small decreases in heritability for cardiac and TPR index from rest to stress in AAs only, which may attributed to the combined effect of dampening of the common genetic factor from rest to stress level and the emergence of new genetic factors for these two variables **(Table 1).**

Again, this study provides clear implications for gene finding studies. The challenged BP and HR levels (e.g., under mental stress in this study) show newly emerging genetic influences that can only be identified if these phenotypes are measured under stress. Thus, we suggest that future studies should use bi- or multivariate designs on resting and aggregated stress levels. Also, we expect that performing gene-by-stress interaction analyses in future gene finding studies will be a promising way forward for detecting genes underlying BP regulation.

Modifying Effect of Obesity on Heritability of BP

Another interesting finding in this thesis is that we found that genetic and environmental influences on SBP vary as a function of general obesity measured as body mass index (BMI) described in **Chapter 7 (Table 1)** (41). The influence of common and unique environmental factors increases with increasing levels of BMI, resulting in a decreased level of heritability. This result seems to suggest that higher BMI levels may reduce the penetrance of genetic vulnerability to SBP through a larger impact of environmental effects. Our results are consistent with those from McCaffery's study investigating effects of educational attainment (42), showing reduced heritability in the high-risk environment using models similar to the present study. That is, SBP heritability was reduced with higher BMIs in our study and lower education levels in McCaffery's. Although the specific mechanism by which BMI affects the heritability of BP cannot be determined from this study, it is well known that several environmental and behavioral

factors that predict BP levels, such as unhealthy diets and lack of physical activity, are more prevalent among groups with higher BMIs. Within the twin design, this type of effect may manifest itself as an enhancement of the environmental effect relative to the genetic effect, resulting in reduced heritability.

Methodological Considerations: candidate gene vs twin studies

In this thesis, we utilized several methods for exploring GxE interaction. This section gives a brief overview of the different approaches used.

Any of the standard epidemiological designs for studying the main effects of genes or environmental factors — cohort designs, case–control designs or hybrid designs, such as nested case–control designs or case–cohort designs — can also be applied to the study of G×E interactions. The issues for choosing between the designs are similar for main effects and interactions, and include the control of confounding and other biases, the temporal sequence of exposure and disease, data quality, the ability to examine multiple end points, and the efficiency of detecting rare diseases or rare risk factors (43). In **Chapter 2**, we applied a prospective design to explore influences of candidate genes, passive smoking and their interaction on infant birth weight. The advantage for this design can be succinctly summarized as the freedom from most biases, and clear temporal sequence of cause and effect; while the disadvantages are: 1) large cohorts and/or long follow-up needed to obtain sufficient numbers of cases; 2) possible biased losses to follow-up; 3) changes in exposure may require recurring observations (43).

From **Chapter 3** to **Chapter 7**, the twin design was applied to explore the influences of gene and environment as well as G x E interaction using different types of twin modeling. Twin studies provide a first necessary step in genetic research by establishing that genes contribute to the observed population variation in risk factors of cardiovascular and other chronic diseases and by estimating the size of this genetic contribution relative to other factors that create resemblance within families. By comparing concordance rates for disease or correlations for continuous traits between monozygotic and dizygotic twins, twin studies can be used to partition components of variance between genetic and shared and non-shared environmental factors (44). Twin studies do not identify the actual genes as this requires molecular genetic research on the measured genetic variants (45). However, they do allow exploration of potential overall G×E effects before specific genes have been identified (43), which may provide clues for subsequent gene-finding studies.

However, the majority of twin studies do not include accurately measured information on environmental exposures that could be shared (or different) between the twins, precluding any inferences about specific gene–environment interactions (44). In **Chapter 7** of this thesis, we performed G x E interaction twin modeling as described by Purcell (46). This model allows investigating whether genetic susceptibility changes as a function of a measured environmental exposure expressed as discrete or continuous variables. We observed in a Chinese population that both common and unique environmental influences on SBP increased with increasing levels of BMI, resulting in a lower heritability at higher BMI levels.

Implications and Future Perspectives

The results in this thesis have implications for further efforts to find genes underlying risk factors (e.g., infant birth weight, BP, intermediate asthma phenotypes, and NE/E) for asthma and cardiovascular disease. Taking BP as an example, even though BP shows substantial heritability of 40-60% (47) , the findings for candidate genes have been difficult to consistently replicate. A recent meta-analysis of GWAS data evaluated associations between 2.5 million genotyped or imputed single nucleotide polymorphisms (SNPs) and SBP and DBP from 29 studies, and found that 29 independent SNPs at 28 loci including 16 novel loci were significantly associated with SBP, DBP, or both (48). However, all the variants identified collectively only explain 0.9% of the phenotypic variance for SBP and DBP (48). This means that the majority of the BP heritability is still "missing" and remains at large. Finding suitable answers to the missing heritability enigma is currently the most important challenge in BP and hypertension genetics. Wang et al. suggested that future studies in BP genetics need to both build on and move beyond the successes of GWAS to make continued progress, for example, by finding causal variants, focusing on rare variants, expanding the search from clinic to ambulatory BP and considering epigenetics (49, 50). Another potential explanation for the difficulty to find genes for BP is that the expression of genes may vary as a function of environmental exposures such as mental stress and BMI in our studies. This would mean their effects can only be found in the presence of certain environments. Future GWA studies may want to rigorously measure environmental exposures and perform GxE interaction analyses to enhance chances of gene finding (51).

Future needs for the study of GxE interactions include the following strategies:

1) Increase the power of analyses for common diseases by studying larger cohorts over a longer time (44). Studying GxE interactions typically requires large populations because analyses need to be stratified by both genotype and environmental exposure. Obviously, collecting large populations renders such studies more difficult and expensive. Pooling data from several cohort studies may help but may also create problems of interpretation if heterogeneity exists in genetic background and/or environmental exposures (16).

2) Accurate measurement of exposures that vary over time or are modifiable by other factors, such as time of exposure, has proven difficult and can create biases in the analysis. Another important issue is the observed heterogeneity in the study design that arises due to differences in the way that examined environmental exposures are assessed across studies and due to the possible study-specific characteristics of exposure (43).

3) The need for integration of environment, genetics and epigenetics in the same study should also be considered, as this could provide insight into their complex interactive role in the establishment of disease (52). In the post-GWAS era, the careful design of epidemiologic studies, accurate measurement of exposures and use of standardized methods across studies should facilitate collaborations, which will increase statistical power for assessing GxE interaction.

Coordinating continuing and future studies to ensure maximum compatibility of the genetic and environmental information obtained is also pivotal for the future GxE interaction studies (44). Virtually all the GxE interactions known to date have been identified through hypothesis-driven research and candidate gene approaches (16). GWAS for common diseases have been successful at identifying novel variants in unexpected genes, but they have come up mostly with common variants of modest effects, which typically explain only a small fraction of the heritability (53). Because both GxE interactions and GWAS appear to be with us to stay, several groups are already busy developing novel analytical methods aimed at allowing efficient testing for GxE interactions in GWAS (54-56). Further GxE interaction studies that are carefully designed can extend the list of genetic loci that exert effects in the presence of specific environmental exposures. The next generation of studies incorporating genetic–environment–epigenome information and utilizing new analytical approaches and environmental measurement technologies can improve understanding of the complex causes of diseases such as asthma and hypertension.

Table 1. Key twin study findings on risk factors for asthma and cardiovascular disease

Chapter	Modeling	Population	Age, mean (years)	Phenotype	Best fitting model	Heritability h^2 (95% CI)			
Chapter 3	Univariate	Caucasians	22.5	PC_{20}	AE	0.47 (0.17-0.70)			
				SPT	AE	0.56 (0.27-0.77)			
				Specific IgE	AE	0.60 (0.31-0.80)			
Chapter 4	Univariate	EA & AA	18.7	$U_{NE}V$	AE	0.68 (0.50-0.79)			
				U_EV	AE	0.74 (0.59-0.83)			
Chapter 5	Univariate	Caucasians	17-62			**Reactivity**			
						Mental stress		Cold pressor	
				SBP	AE	M: 0.26 (0.23-0.29) F: 0.38 (0.33-0.43)		ACE	M: 0.21 (0.04-0.35) F:0.33 (0.28-0.40)
				DBP	ADE	A: 0.14 (0.08-0.20) D: 0.15 (0.07-0.24)		AE	0.55 (0.50-0.61)
				HR	AE	0.43 (0.40-0.47)		AE	0.45 (0.40-0.51)

Chapter	Modeling	Population	Age, mean (years)	Phenotype	Best fitting model	Heritability h^2 (95% CI)		
						Reactivity	Stress level	Due to genes emerging during stress
Chapter 6	Bivariate	EA	14.8	SBP	AE	0.48(0.37-0.57)	0.68(0.60-0.75)	0.25(0.19-0.31)
				DBP	AE	0.38(0.27-0.47)	0.67(0.58-0.73)	0.28(0.20-0.35)
				HR	AE	0.42(0.29-0.53)	0.70(0.64-0.76)	0.12(0.08-0.16)
				SV	AE	NS	0.53(0.42-0.62)	NS
				Cardiac index	AE	NS	0.51(0.41-0.59)	NS
				TPR index	AE	0.21(0.09-0.33)	0.55(0.46-0.64)	0.08(0.04-0.13)
		AA	14.5	SBP	AE	0.50(0.37-0.60)	0.72(0.63-0.79)	0.24(0.17-0.31)
				DBP	AE	0.37(0.24-0.50)	0.73(0.65-0.80)	0.22(0.14-0.29)
				HR	AE	0.34(0.18-0.48)	0.68(0.58-0.75)	0.10(0.05-0.15)
				SV	AE	0.28(0.11-0.42)	0.58(0.45-0.67)	0.06(0.02-0.10)
				Cardiac index	AE	0.35(0.20-0.49)	0.31(0.14-0.45)	0.08(0.03-0.12)
				TPR index	AE	0.34(0.18-0.48)	0.39(0.23-0.52)	0.10(0.05-0.16)
Chapter 7	GxE model	Chinese Han	37.8	SBP	ACEU VB	Lower heritability at higher BMI levels; Heritability was 14 percentage points lower for SBP (P<0.001) among overweight twins ($h^2 = 0.31$) relative to normal weight twins ($h^2 = 0.45$).		
				DBP	ACEB	No interaction detected ($h^2 = 0.26$)		

EA: European American; AA: African American; M: male; F: female; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SV: stroke volume; TPR: total peripheral resistance; BMI: body mass index; PC_{20}: the provocation concentration producing a 20% fall in FEV_1; SPT: skin prick test; $U_{NE}V$: overnight urinary excretion rate of norepinephrine; U_EV: overnight urinary excretion rate of epinephrine; h^2: heritability; A: additive genetic effects; D: dominant genetic effects; C: shared environmental effects; E: non-shared environmental effects; U: moderated component of C; V: moderated component of E; B: linear effects of moderator on mean; CI: confidence interval; NS: not significant

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Summary

The search for genetic factors that influence common, complex traits and the characterization of the effects of those factors is both a goal and a challenge for modern geneticists. In the last couple of years, gene-finding has been revolutionized by the success of genome-wide association studies (GWAS), which have identified hundreds of genetic variants associated with complex human diseases and traits, and have provided valuable insights into their genetic architecture. However, most variants identified so far confer relatively small increments in risk, and explain only a small proportion of phenotype variance, leading many to question how the remaining, 'missing' heritability can be explained. This unexplained genetic variation may be due to low frequency alleles or other types of variation not captured by current GWAS techniques and/or to underdeveloped data analysis methods for detecting complex interactions. The detection of gene-environment (GxE) interactions is important because if a genetic factor operates primarily through a complex mechanism involving multiple other genes, and possibly environmental factors, the effect may be missed if one examines it in isolation, without allowing for its potential interactions with these other (unknown) factors. The main aim of this thesis was to apply different GxE interaction methods on risk factors for asthma and cardiovascular disease, to explore their strengths and limitations and stimulate future applications.

In this thesis, we used both a candidate gene design, in which DNA is measured, and a twin design, in which DNA is unmeasured, to explore GxE interactions in different chapters. In **chapter 2**, we aimed to explore whether polymorphisms in two maternal metabolic genes, cytochrome P-450 1A1 (*CYP1A1*) *MspI* and epoxide hydrolase 1 (*EPHX1*) Tyr113His, affect the association of maternal passive smoking with infant birth weight in a cohort of 1388 newly married mothers in China. Results showed that variants of metabolic enzyme genes *CYP1A1* and *EPHX1* modified the association between maternal passive smoking and infant birth weight in the Chinese population. In the passive smoking group, there was a remarkably lower birth weight for the *CYP1A1* MspI C/C6235 compared to the T/T6235 genotype (156.3 g) and for both the *EPHX1* Tyr/His113 (93.8 g) and the His/His113 (244.6 g) compared to the Tyr/Tyr genotype. The *CYP1A1* MspI and *EPHX1* genotypes modified the association between maternal passive smoking and infant birth weight in this study, indicating GxE interaction. Gene-maternal smoking interaction studies are of increasing importance in investigating fetal growth retardation and low birth weight. Although smoking

cessation and avoidance of exposure to passive smoking should be advised to all pregnant women, a genetic risk profile might help us to target more effectively those at higher risk of growth retardation and adverse pregnancy outcomes.

The remaining five chapters of the thesis consisted of a number of twin studies with the purpose to extend applications of twin modeling to explore effects of GxE interactions on intermediate phenotypes for asthma and cardiovascular disease. In **chapter 3**, we showed that the provocation concentration producing a 20% fall in FEV1 (PC20), skin prick test (SPT) and specific Immunoglobulin E (IgE), which are intermediate asthma phenotypes reflecting direct responses to environmental stimuli such as methacholine and 11 common allergens have moderate heritabilities of 0.47, 0.56 and 0.60, respectively. Much of the phenotypic correlations between these objective asthma related traits are attributable to genetic factors (most were above 70%). At the same time remarkable environmental overlap (0.70) was seen between specific IgE and SPT in our study, perhaps because these traits represent responses to similar allergens. What deserves to be mentioned is that SPT and PC20 also showed a considerable degree of environmental overlap (0.44). These findings widen the insight of the origins of clinical homogeneity for asthma-related traits and provide some clues and directions for gene finding studies

It is reported that the catecholamines epinephrine (E) and norepinephrine (NE) mediate the stress response via the sympathetic nervous system and may play a key role in homeostatic blood pressure (BP) control. In the study described in **Chapter 4**, we observed significant heritabilities for overnight urinary excretion rates of NE ($U_{NE}V$, 0.68) and E (U_EV, 0.74) as measures of chronic stress exposure, in both African Americans and European Americans from the Georgia Cardiovascular Twin Study, without ethnic and gender effects. We also observed a high genetic correlation of 0.86 between $U_{NE}V$ and U_EV. These results suggest that genetic factors are strong determinants of basal sympathetic activity.

In **chapter 5**, we performed a meta-analysis on all published twin studies that assessed heart rate (HR) or BP reactivity to the cold pressor test or various mental stress tasks. Moreover, we also briefly discussed heritability estimates of a number of other cardiovascular measures for which sufficient numbers are not yet available to do a meta-analysis. We further reviewed the first attempts to find genetic associations with reactivity measures in molecular genetic studies. For systolic BP (SBP) reactivity to the mental stressors, the pooled heritability across all studies ranged from 0.26 (males) to

0.38 (females). SBP reactivity to the cold pressor test yielded comparable heritability estimates ranging from 0.21 (males) to 0.33 (females). For diastolic BP (DBP) reactivity, heritability for the cold pressor test was higher (0.55) than that for mental stress even after including dominance variation (broad heritability of 0.29). Heritability estimates for HR reactivity to mental stress (0.43) and the cold pressor test (0.45) were very similar. However, a limitation of most twin studies performed so far, and hence of the meta-analysis based on these studies, is that they analyzed reactivity as a change score. That way, the heritability estimates will reflect an inseparable mix of both newly emerging genetic or environmental influences during stress and an amplification or dampening of genetic or environmental influences already present at rest. Thus in **chapter 6**, we further performed bivariate modeling of resting and stress levels to explicitly test for emergence and amplification.

In **Chapter 6**, we replicated the work by De Geus et al. in adolescents and extended it to another ethnic group by including not only EA but also a group of AA twins. Furthermore, we extended it to include cardiac output (CO) and total peripheral resistance (TPR) of the systemic vasculature as hemodynamic determinants of BP. We observed that heritability indices for levels at rest and during stress were high (most were above 50%) and comparable between ethnic groups. We observed increases in heritability for BP and HR from rest to stress that were mostly explained by newly emerging genetic influences on the added stress component. The stable component of the heritability was accounted for by a simultaneous decrease in genetic and environmental variances. Again, this study provides clear implications for gene finding studies. The challenged BP and HR levels (e.g., under mental stress in this study) show newly emerging genetic influences that can only be identified if these phenotypes are measured under stress. Thus, we suggest that future studies should use bi- or multivariate designs on resting and stress levels. Also, we expect that performing gene-by-stress interaction analyses in future gene finding studies will be a promising way forward for detecting genes underlying BP regulation.

Another interesting finding in this thesis is that genetic and environmental influences on SBP vary as a function of general obesity, measured as body mass index (BMI), in Han Chinese twins from the Chinese National Twin Registry (CNTR) as described in **Chapter 7**. The influence of common and unique environmental factors increases with increasing levels of BMI, resulting in a decreased level of heritability. This result seems to suggest that higher BMI levels may reduce the penetrance of genetic vulnerability to

SBP through a larger impact of environmental effects. Although the specific mechanism by which BMI affects the heritability of BP cannot be determined from this study, it is well known that several environmental and behavioral factors that predict BP levels, such as unhealthy diets and lack of physical activity, are more prevalent among groups with higher BMIs. Within the twin design, this type of effect may manifest itself as an enhancement of the environmental effect relative to the genetic effect, resulting in reduced heritability.

In **Chapter 8**, the main findings of this thesis are summarized and their interpretation and methodological considerations given. Furthermore, the implications and future perspectives are discussed. The advantage for the candidate gene design can be succinctly summarized as the freedom from most biases, and clear temporal sequence of cause and effect; while there is also some disadvantages as described in this chapter. On the other hand, twin studies do not identify the actual genes as this requires molecular genetic research on the measured genetic variants. However, they do allow exploration of potential overall G×E effects before specific genes have been detected, which may provide clues for subsequent gene-finding studies. Overall, the findings presented in this thesis have implications for further efforts to find genes underlying risk factors for asthma and cardiovascular disease (e.g., infant birth weight, BP, intermediate asthma phenotypes, and NE/E). To conclude, I provide my perspective on future GxE interaction research in terms of using larger study populations, accurate measurements of exposures, integration of the environment, genetics and epigenetics, and maximum compatibility of the genetic and environmental information obtained.

Samenvatting

De zoektocht naar en het vaststellen van het effect van erfelijke factoren die veelvoorkomende complexe eigenschappen beïnvloeden is zowel een doel als een uitdaging voor de moderne genetica. De laatste jaren is het vinden van genen in een stroomversnelling geraakt door het succes van genoombrede associatie studies (GWAS) die honderden genetische varianten voor complexe ziekten en eigenschappen hebben geïdentificeerd en waardevolle inzichten in hun erfelijke opbouw hebben gegeven. De meeste varianten die tot nu toe zijn gevonden hebben echter slechts een kleine toename in het risico tot gevolg en verklaren slechts een klein deel van de fenotypische variantie, wat velen ertoe gebracht heeft zich af te vragen waar het resterende deel van de erfelijkheidsschatting (de zgn. "missing heritability") gevonden kan worden. Deze onverklaarde erfelijke variantie kan wellicht toegeschreven worden aan zeldzame of andersoortige genetische varianten die niet door de huidige GWAS genotyperingschips worden gemeten en/of aan onderontwikkelde data analyse methoden voor het ontdekken van complexe interacties. De detectie van gen-omgeving (GxE) interacties is belangrijk aangezien een genetische factor voornamelijk kan werken door middel van een complex mechanisme waarbij meerdere genen en omgevingsfactoren een rol spelen en het effect gemist kan worden als het op zichzelf bestudeerd wordt zonder rekening te houden met mogelijke interacties met deze andere (onbekende) factoren. Het belangrijkste doel van dit proefschrift was dan ook om verschillende GxE interactie methoden toe te passen op risicofactoren voor astma en hart- en vaatziekten om zo hun sterke en zwakke punten in kaart te brengen en toekomstige toepassingen te stimuleren.

In dit proefschrift hebben we zowel een kandidaatgen design gebruikt, waarin DNA wordt gemeten, als een tweeling design, waarin het DNA ongemeten blijft, om GxE interacties te onderzoeken in de verschillende hoofdstukken. In **hoofdstuk 2**, bekeken we of polymorphismen in twee maternale metabole genen, cytochroom P-450 1A1 (*CYP1A1*) *MspI* en epoxide hydrolase 1 (*EPHX1*) Tyr113His, de associatie van passief roken van de moeder met geboortegewicht van het kind beïnvloedt in een cohort van 1388 pas getrouwde moeders in China. Resultaten lieten zien dat varianten van deze metabole enzym genen *CYP1A1* en *EPHX1* het verband tussen passief roken van de moeder en geboortegewicht van het kind modificeerde in de Chinese populatie. In de passief roken groep was er een opmerkelijk lager geboortegewicht in de groep met het *CYP1A1* MspI C/C6235 vergeleken met het T/T6235 genotype (156.3 g) en voor zowel

de *EPHX1* Tyr/His113 (93.8 g) als de His/His113 (244.6 g) vergeleken met de Tyr/Tyr genotype groep. De *CYP1A1* MspI and *EPHX1* genotypes modificeerden het verband tussen passief roken van de moeder en geboortegewicht van het kind in deze studie wat duidt op GxE interactie. Studies naar de interactie tussen genen en roken van de moeder zijn van toenemend belang om het verband te begrijpen tussen groeivertraging van de foetus en laag geboortegewicht. Hoewel stoppen met roken en vermijden van blootstelling aan passief roken geadviseerd zou moeten worden aan alle zwangere vrouwen, zou een genetisch risicoprofiel ons kunnen helpen gerichter advies te geven aan moeders met een hoger risico op groeivertraging in het kind en daaraan gerelateerde ongunstige zwangerschapsuitkomsten.

De resterende vijf hoofdstukken van het proefschrift bestonden uit een aantal tweelingstudies met het doel tweelingmodellen uit te breiden om effecten van GxE interacties op tussenliggende fenotypen voor astma en hart- en vaatziekten te onderzoeken. In **hoofdstuk 3**, hebben we laten zien dat de provocatie concentratie die een 20% reductie in FEV1 produceert (de zgn. PC20), de "skin prick test (SPT)" en specifiek Immuunglobuline E (IgE), allen tussenliggende astma fenotypen die een directe respons op omgevingsstimuli weergeven zoals methacholine en 11 veelvoorkomende allergenen, middelmatig grote erfelijkheidsschattingen van respectievelijk 0.47, 0.56 en 0.60 hebben. Veel van de fenotypische correlaties tussen deze objectieve astma gerelateerde eigenschappen zijn toe te schrijven aan erfelijke factoren (meestal meer dan 70%). Tegelijkertijd was er een opmerkelijke omgevingsoverlap (0.70) tussen specifiek IgE en SPT in onze studie, misschien omdat deze maten reacties op vergelijkbare allergenen representeren. Verder lieten ook SPT en PC20 een substantiële mate van omgevingsoverlap zien (0.44). Deze bevindingen geven inzicht in de oorsprong van klinische homogeniteit voor astma gerelateerde trekken en geven enkele eerste aanwijzingen en mogelijke richtingen voor studies die als doel hebben nieuwe genen voor deze aandoeningen te vinden.

Het is bekend dat de catecholaminen adrenaline (A) en noradrenaline (NA) de stress-respons mediëren via het sympathische zenuwstelsel en een sleutelrol spelen in de bloeddruk (BD) regulatie. In the studie beschreven in **Hoofdstuk 4** observeerden we significante erfelijkheidsschattingen voor nachtelijke urine uitscheidingssnelheid van NA ($U_{NA}V$, 0.68) en A (U_AV, 0.74) als maten voor chronische blootstelling aan stress, in zowel Afro-Amerikanen als Europese Amerikanen van de Georgia Cardiovascular Twin Study, zonder effecten van etniciteit en geslacht. We vonden ook een hoge genetische

correlatie van 0.86 tussen $U_{NA}V$ en U_AV. Deze resultaten wijzen erop dat erfelijke factoren sterke determinanten van basale sympathische activiteit zijn.

In **hoofdstuk 5**, hebben we een meta-analyse uitgevoerd op alle gepubliceerde tweelingstudies die hartslag en BD reactiviteit op de zgn. cold pressor test en verscheidene mentale stress taken hadden gemeten. Bovendien gaven we een korte discussie van de erfelijkheidsschattingen van een aantal andere cardiovasculaire maten waarvoor nog onvoldoende data beschikbaar was om een meta-analyse te kunnen doen. Verder gaven we een overzicht van de eerste pogingen om genetische associaties te vinden met reactiviteitsmaten in moleculair genetische studies. Voor systolische BD (SBD) reactiviteit op de mentale stressoren, the gepoolde erfelijkheidsschatting over alle studies varieerde van 0.26 (mannen) tot 0.38 (vrouwen). SBD reactiviteit op de cold pressor test gaf vergelijkbare erfelijkheidsschattingen variërend van 0.21 (mannen) tot 0.33 (vrouwen). Voor diastolische BD (DBD) reactiviteit, de erfelijkheidsschatting voor de cold pressor test was hoger (0.55) dan die voor mentale stress zelfs na inclusie van dominantie variantie ("broad-sense heritability" van 0.29). Erfelijkheidsschattingen voor hartslag reactiviteit op mentale stress (0.43) en de cold pressor test (0.45) waren vergelijkbaar. Echter, een beperking van de meeste tweelingstudies die tot nu toe zijn uitgevoerd, en dus van de meta-analyses gebaseerd op deze studies, is dat ze reactiviteit als een verschilscore hebben geanalyseerd. Op die manier reflecteren de erfelijkheidsschattingen van de reactiviteit een onontwarbare mix van zowel nieuw tot expressie komende genetische of omgevingsinvloeden tijdens de stress (emergentie) als ook een amplificatie (of afzwakking) van genetische of omgevingsinvloeden die al aanwezig zijn in rust. Daarom hebben we in **hoofdstuk 6** bivariate modellen toegepast op zowel rust als stress niveaus om expliciet te kunnen testen voor emergentie en amplificatie.

In **Hoofdstuk 6**, repliceerden we het werk van De Geus et al. in adolescenten en breidden het uit naar een andere etnische groep door niet alleen Europese Amerikanen maar ook een groep Afro-Amerikanen te includeren. De studie werd verder uitgebreid met hartminuutvolume en totale perifere weerstand van het vaatstelsel als hemodynamische determinanten van BD. We vonden dat erfelijkheidsschattingen voor rust en stress niveaus hoog waren (meestal boven de 50%) en vergelijkbaar tussen etnische groepen. Verder zagen we toenames in erfelijkheidsschattingen voor BD en hartslag van rust naar stress die voornamelijk verklaard konden worden door nieuwe (emergente) genetische invloeden op de stress component. De stabiele component van

de erfelijkheidsschattingen werd veroorzaakt door een gelijktijdige afname in genetische en omgevingsvarianties. Deze studie biedt opnieuw duidelijke implicaties voor het vinden van nieuwe genen. De BD en hartslag niveaus onder mentale stress in deze studie laten nieuwe emergente erfelijke invloeden zien die slechts geïdentificeerd kunnen worden als deze eigenschappen onder stress worden gemeten. Daarom raden we aan dat toekomstige studies bi- en multivariate designs gebruiken om zowel rust als stress niveaus te onderzoeken. Bovendien verwachten we dat het uitvoeren van gen-stress interactie analyses in toekomstige studies een veelbelovende manier zal zijn om genen te detecteren die aan BD regulatie ten grondslag liggen.

Een andere interessante bevinding in dit proefschrift is dat genetische en omgevingsinvloeden op SBD variëren als een functie van obesitas, gemeten door middel van body mass index (BMI), in Han Chinese tweelingen van het Chinese Nationale Tweeling Register (CNTR) zoals beschreven in **Hoofdstuk 7**. De invloed van gedeelde en unieke omgevingsfactoren neemt toe met toenemende niveaus van BMI, resulterend in een afname van de erfelijkheidsschatting. Dit resultaat lijkt erop te duiden dat hogere BMI waarden het tot uiting komen van de genetische kwetsbaarheid voor SBD reduceren door een grotere impact van omgevingseffecten. Hoewel het specifieke mechanisme waardoor BMI de erfelijkheidsschatting van BD beïnvloedt niet bepaald kon worden met deze studie, is het bekend dat verscheidene omgevings- en gedragsfactoren die BD voorspellen, zoals een ongezond dieet en te weinig fysieke activiteit, vaker voorkomen in groepen met hogere BMIs. Binnen het tweelingdesign kan zo'n effect zich manifesteren als een vergroting van het omgevingseffect ten opzichte van het genetische effect, wat leidt tot een kleinere erfelijkheidsschatting.

In **Hoofdstuk 8**, worden de belangrijkste bevindingen van dit proefschrift samengevat en interpretaties en methodologische overwegingen gegeven. Verder worden implicaties en toekomstperspectieven geschetst. Het voordeel van het kandidaatgen design kan kort samengevat worden als vrijwaring van de meeste biases en een duidelijke tijdssequentie van oorzaak en gevolg, maar er zijn ook enkele nadelen zoals beschreven in dit hoofdstuk. Aan de andere kant kunnen tweelingstudies niet de daadwerkelijke genen identificeren aangezien hiervoor moleculair genetische studies van de gemeten genetische varianten vereist zijn. Echter, zij staan wel de exploratie van mogelijke algemene G×E effecten toe voordat specifieke genen zijn ontdekt, wat vervolgstudies kan helpen die het vinden van genen tot doel hebben. In het algemeen hebben de bevindingen zoals beschreven in dit proefschrift implicaties voor verdere

pogingen om genen te vinden die ten grondslag liggen aan risicofactoren voor astma en hart- en vaatziekten (bijv.: geboortegewicht, BD, tussenliggende astma fenotypen, en NA/A). Concluderend geef ik mijn perspectief op toekomstig GxE interactie onderzoek waarin vooral grotere studie populaties, nauwkeurige metingen van blootstellingen, integratie van omgeving, genetica en epigenetica, en maximale afstemming van verkregen erfelijke en omgevingsinformatie belangrijk zullen zijn.

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吴婷 2013 年 8 月

About the author

Curriculum Vitae

Ting Wu was born in Beijing, China on the 23th of November 1980. After obtaining her M.D. in Preventive Medicine from Peking University Health Science Center in 2004, she started her first PhD project in the Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, mainly focused on genetic and cardiovascular disease epidemiology. In March 2008, Ting Wu started her second PhD project, in the Unit of Genetic Epidemiology & Bioinformatics, Department of Epidemiology, University Medical Center Groningen, the Netherlands, under the supervision of Prof. Harold Snieder. Her PhD research project investigated the influence of genes, environment and their interaction on risk factors for asthma and cardiovascular disease using data from the Georgia Cardiovascular Twins Study, the Chinese National Twin Registry, the Prospective Reproductive Health Study among Chinese Textile Women Workers project, and the Netherlands Twin Register. In July 2010, Ting Wu obtained her first PhD at the Peking University Health Science Center. She is currently working as a Senior Scientist/Epidemiologist in Biostatistics and Research Decision Sciences-AP (BARDS-AP), Merck Research Laboratories, Beijing, China.

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Education and training

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- Drug Information Association (DIA) Workshop: Global Clinical Trials in Drug Development-Principles and Case Studies (2013, Beijing, China)
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- Twin Model Fitting (Mx) course at the SGDP Centre's 10th Summer School (2009, London, UK)
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- Study Design in Clinical Epidemiology (2008, Groningen, the Netherlands)
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- SNP and Human Diseases Course, Erasmus MC (2008, Rotterdam the Netherlands)
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Scientific presentations

- **WU T** (Invited Speaker). Pharmacoepidemiologic Perspectives on Real World Evidence and Comparative Effectiveness Research. CORE China Outcome Research & Evidence Based Medicine. Shanghai, China, 2013
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